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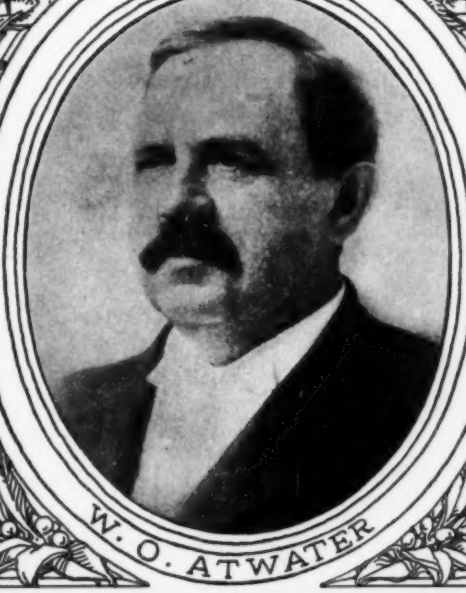

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THE EFFECT OF DIET ON THE VITAMIN A CONTENT OF THE BOVINE FETAL LIVER [1]

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(Received for publication June 10, 1943)

In a recent general review article on vitamin A (Butt, '42), the statement is made that the vitamin A content of the liver is very low at birth, irrespective of the diet of the mother. Hart and Guilbert ('33) made a similar statement for cattle — namely, that "the livers of new-born animals tend to be comparatively low in vitamin A, irrespective of the diet of the dam during gestation". In the same paper they report that livers from four aborted calves whose dams were on a vitamin A-deficient diet contained no vitamin at all. The vitamin A content of livers of new-born and premature children has been investigated by Wolff ('32), Ellison and Moore ('37), Toverud and Ender ('35), Gaethgens ('37), Neuweiler ('36), and With ('40). All, except Gaethgens, found low reserves of vitamin A with considerable individual variation. Their material did not permit correlation of their findings with the diet of the mother. Low reserves were also observed by Debré and Busson ('34) in new-born children and puppies, by Dann ('34) in rats and rabbits, and by V. Eekelen and Wolff ('36) in kittens and new-born puppies. Dann observed no effect on the vitamin A content of the livers of new-born rats and rabbits after the mother had been on a carotene-enriched diet, but found slightly higher values after the mother had been on a vitamin A-enriched diet. These latter values showed no relation to the amount of vitamin A given to the mother.

[1] The U. S. Bureau of Animal Industry has contributed to the cost of this work.

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Recent results obtained in this laboratory with cattle on different dietary regimes indicate that the vitamin A content of fetal livers, although low, is in direct relationship to the mother's diet.

As part of a project to investigate the blood chemistry of vaccinated and nonvaccinated cows on different diets exposed to Brucella, the liver vitamin A of aborted fetuses was determined. The method described by Guilbert and Hart ('34) for estimation of vitamin A in liver tissue was modified for use with the Cenco photometer, using Wratten filter no. 47 for the reading of carotenoids and Wratten filter no. 58 for the reading of the blue color developed after addition of $SbCl_3$ in chloroform to the prepared sample. The calculation for vitamin A and carotenoid correction described by Kimble ('39) was used. The weight of the fresh liver samples was 5 gm. and although the vitamin A content of all fetal livers was very low, an extraction of the saponified material in 20 ml. of special petroleum ether (B.P., 33–36°C.) gave $SbCl_3$ reactions of distinct color, permitting the photometric measurement of differences in this material with low vitamin A content.

The mothers of the fetuses under investigation were maintained in four different dietary groups. The pasture group (P) consisted of animals which remained on non-irrigated natural grass pasture in Strawberry Canyon, Berkeley, California, throughout the year, without supplementary feed. The average blood vitamin A level of these animals was very low from September to January, when no green feed was found on the pasture.

Another group (PA) consisted of animals which were maintained on the same pasture as the P group but were fed 400,000 I.U. of vitamin A, in the form of shark-liver oil, twice a week from September through November. The average blood vitamin A level of these animals was higher than that of the P group.

One group of animals was transferred from pasture into the barn (B group) on September 1st and was maintained

there on a carotene and A-deficient diet consisting of beetpulp, cottonseed meal and rolled barley. A number of the barn animals received a shark-liver oil supplement (400,000 I.U.) twice a week from September through November (BA group). From October on, the average blood vitamin A level of the B group was lower than that of the P group, and the level of the BA group was considerably higher than that of the B group and approximately the same as that of the PA group.

The pregnant animals were exposed to Brucella abortus on October 2nd. Most of the nonvaccinated and a few vaccinated animals aborted during November and December. The values which were found for vitamin A content of the livers of the aborted fetuses are assembled in table 1.

The analysis of variance between the four groups revealed a highly significant difference between their means, with an F value of 11.8, which, for the appropriate degrees of freedom, corresponds to a P level of considerably less than 1%. The significance of differences between any pair of means due to unequal variance in the group may best be judged by the square root of the sum of squared standard errors. This method of analysis indicates that out of six possible comparisons only that involving the PA and BA groups does not reach statistical significance.

These significant differences for the four dietary groups indicate an effect of the dam's diet upon the vitamin A storage of the fetus. Since the vitamin A values of the blood of the fetuses are extremely low (0–10 I.U./100 ml., which is too low to permit reliable measurements of differences among groups), it may be assumed that only a very small fraction of the dam's vitamin A is able to pass through the placenta. However, these small amounts as reflected in the liver levels are in direct relationship to the dam's vitamin A storage. This is further substantiated by a few tests in which the vitamin A content of the dam's liver could be determined. The junior author performed a number of operations in which he removed the caudate lobe of the dam's liver under para-

TABLE 1

The vitamin A content of the fetal livers arranged in groups according to the diet of the dam. The vitamin A content of the liver of the dam in the four dietary groups.

COW NO.	LIVER FROM DAM	GESTATION PERIOD	LIVER FROM FETUS
	I. U./gm.	*days*	*I. U./gm.*
	B Group (Barn fed, low A diet)		
319	120.8	261	2.7
276	....	210	0.0
2	....	238	3.0
313	....	226	2.5
343	94.0	226	0.0
312	....	212	3.9
200	96.8	277 (calf still-born)	0.0
		Mean	1.7 ± 0.58
		Median	2.5
	BA Group (Barn fed, plus shark-liver oil)		
324	....	251	24.2
282	335.8	221	10.1
247	....	226	10.3
293	....	245	15.9
318	....	258	9.8
		Mean	14.1 ± 2.48
		Median	10.3
	P Group (Pasture fed)		
315	....	230	7.4
263	244.7	232	6.2
305	....	242	6.1
3	195.8	...	
		Mean	6 6 ± 0.34
		Median	6.2
	PA (Pasture fed, plus shark-liver oil)		
273	449.4 [1]	233	9.5
274	....	210	6.8
326	342.9 [1]	220	8.5
296	....	261	15.1
4	....	180	13.7
		Mean	10.7 ± 1.41
		Median	9.5

[1] Data from animals slaughtered in January, 4 weeks after abortion.

vertebral anesthesia. Partial hepatectomy was done within 24 hours after abortion, thus yielding samples which could be compared with the liver sample of the fetus (table 1).

Using the median values of the data presented in table 1, relationships between the vitamin A values for each pair of groups were obtained for the dams and fetuses (table 2). These relationships suggest the possibility of obtaining more data on this subject from slaughterhouse material. It would be unnecessary to know the past nutritional history of the animal — the simultaneous determination of the vitamin A content of the mother's liver and the fetus' liver would suffice to establish relationships as demonstrated above. Fetuses from

TABLE 2

Relationship between the vitamin A values of dams and fetuses.

GROUP			RATIO OF VITAMIN A AMONG MOTHERS	RATIO OF VITAMIN A AMONG FETUSES
Barn fed	:	Barn fed plus shark-liver oil	1: 3.5	1: 4.1
Barn fed	:	Pasture fed	1: 2.3	1: 2.5
Barn fed	:	Pasture fed plus shark-liver oil	1: 4.1	1: 3.8
Pasture fed	:	Barn fed plus shark-liver oil	1: 1.5	1: 1.7
Pasture fed plus shark-liver oil	:	Barn fed plus shark-liver oil	1: 0.9	1: 1.1
Pasture fed	:	Pasture fed plus shark-liver oil	1: 1.8	1: 1.5

mothers with similar liver vitamin A levels should show a similar vitamin A content in the liver, and the vitamin A stores of mothers and fetuses should be proportionately large or small.

Wolff ('32), Toverud and Ender ('35), and With ('40) have demonstrated that vitamin A values of children born at term do not differ from those of premature children. If such is the case in cattle also, it should be stated that the differences observed here in fetal livers from mothers with different vitamin A stores are much too small to have an effect on the welfare of the new-born normal calf; however, the differences are large enough to reflect the vitamin A status of the mother.

SUMMARY

During an experiment in which cattle were exposed to Brucella abortus, pregnant cows were separated into four dietary groups with different vitamin A intake. The vitamin A content of the livers of twenty fetuses aborted by these cows was measured. At the same time, the vitamin A storage of a few mothers in each dietary group was determined from liver samples obtained by partial hepatectomy immediately after abortion. It was found that the vitamin A content of the fetal liver, although low, was in direct relationship to the mother's diet.

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THE PROPHYLACTIC REQUIREMENT OF THE RAT FOR ALPHA TOCOPHEROL[1]

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TWO TEXT FIGURES AND ONE PLATE (FOUR FIGURES)

(Received for publication July 7, 1943)

Quantitative studies on the actual amount of tocopherol prophylactically required for all normal phenomena should be carried out for a significant portion of the life span of an animal or at any rate throughout early adult life. The requirement may be judged to be lower than is actually the case if an inevitable storage of tocopherol occurs in the tissues of normal young during intrauterine life and the first 3 weeks of postnatal existence. This study undertakes to determine the minimal daily requirement of the rat for tocopherol throughout early adult life.

Mason ('40) has carefully studied the alpha tocopherol requirement of male and female rats during the early months of life. Mothers and young were placed on a vitamin E low diet during the last week of lactation. The young were thus restricted to the vitamin E conveyed by placental and mammary transfer. The weaned young were continued on the vitamin E low diet for an additional 10 days before supplements of alpha tocopherol were given. The males were sacrificed after experimental periods of 50 to 90 days and the

[1] Aided by grants from the Board of Research and from the Department of Agriculture of the University of California, and the Rockefeller Foundation, New York City. The following materials were generously contributed: yeast by The Vitamin Food Company of New York, and Ephynal, by Hoffman-LaRoche Company, Nutley, New Jersey.

testes and epididymis were studied histologically. A comparison was made with testes and epididymis which had been removed at the time of expected degeneration (after 40 to 70 days on the vitamin E deficiency diet). Females were mated with normal males after attaining a weight of 150 gm. (average experimental period 45 days) and the administration of alpha tocopherol was continued through the tenth day of pregnancy. In most instances a laparotomy was performed on the sixteenth day and the number of living fetuses observed. In some cases the females were allowed to continue until term. Under these conditions the minimal daily requirement for alpha tocopherol appeared to lie between 0.0375 and 0.0750 mg. He concluded that the needs of the two sexes were essentially the same. Lactation in the female and reproductive performance in the male were not studied.

Evans, Emerson and Emerson ('39), showed that a daily dose of 0.75 mg. of alpha tocopherol protected against sterility in the male to the age of 9 months; the present study extends this period to 16 months.

The extensive experiment herein reported was designed to establish the daily need for alpha tocopherol in the case of both sexes from weaning until somewhat before the approach of senescence. The study included growth, condition of the striated musculature, reproductive performance, and the prophylactic requirement of suckling young for tocopherol as measured by the presence or absence of muscular dystrophy.

EXPERIMENTAL

Twenty-one-day-old male and female rats were placed either on our standard vitamin E low ration (Diet 427 [2]) or on Diet I + lettuce at 21 days. These young were weaned from

[2] Diet 427

Commercial casein	27	Brewer's yeast	10
Cooked corn starch	35	McCollum Salts No. 185	4
Lard	22	Cod liver oil	2

Diet without cod liver oil is let stand for 2 weeks to accelerate rancidity changes in the lard; the cod liver oil is added just before using.

stock colony mothers maintained on a natural food ration (Diet I + lettuce [3]).

Littermate brothers and littermate sisters were segregated into five groups, one of which was continued on the natural food ration (Diet I and lettuce); four groups on Diet 427 received six times weekly 40 mg. ethyl laurate or 0.10 mg., 0.25 mg., and 0.75 mg. α-tocopheryl acetate dissolved in 40 mg. ethyl laurate respectively.

Ten males and thirty females were started in each group. Fifteen females were used for the reproduction studies and fifteen were employed for growth.

The male rats were offered oestrus females at fortnightly to monthly intervals from the age of 4 to 16 months.

The requirement of the male rat for alpha tocopherol

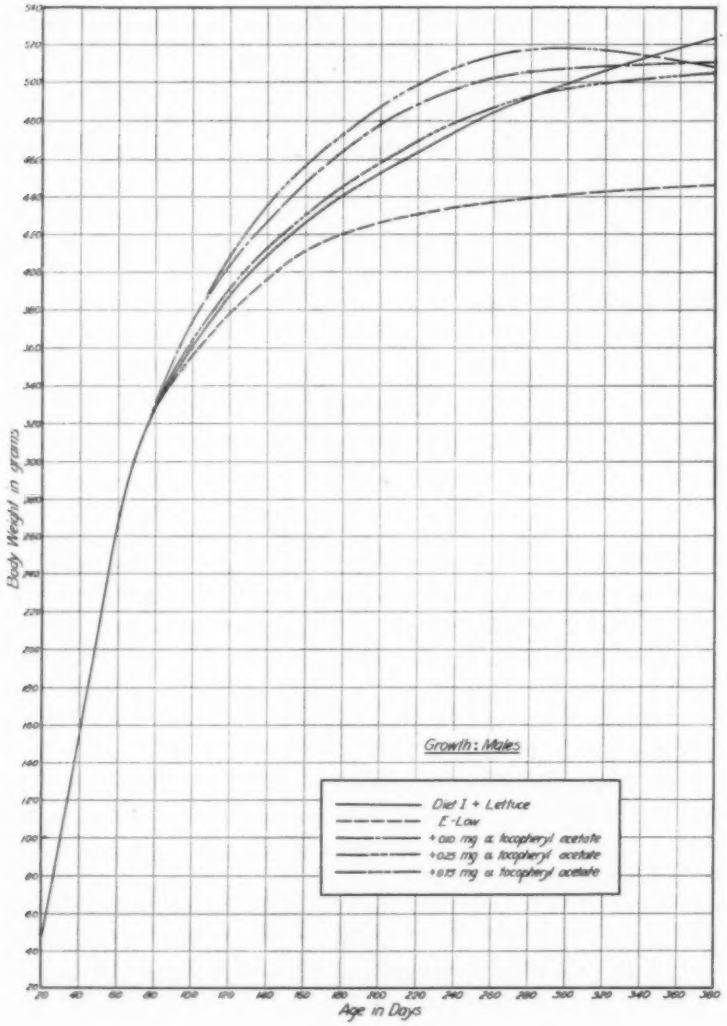
The vitamin E low males showed a plateau in the weight curves at the age of approximately 6 months (graph 1). The rats receiving all three levels of alpha tocopheryl acetate were equal to or superior in growth to those receiving the stock rations. A careful examination of the gastrocnemius muscle at autopsy on the seventeenth month showed no instances of microscopic lesions in the musculature even in the group receiving only 0.1 mg. tocopherol daily. No evidences of motor impairment were ever seen in these animals. On the other hand, the E low animals were both "clinically" and microscopically impaired.

All vitamin E low rats were sterile after the second mating (4½ months, tables 1 and 2). This initial fertility can be attributed to storage as can be attested by the subsequent sterility noted for the group.

Five of the ten animals on the lowest level of tocopherol (0.10 mg.) were sterile after the second mating; however, three of the rats in this group were fertile until the age of

[3] Diet I + lettuce ad libitum

Ground whole wheat	67.5	Melted butter	5
Casein	15	Calcium carbonate	1.5
Whole milk powder	10	Sodium chloride	1


Body Weight in grams
Age in Days
Growth: Males
Diet I + Lettuce
E-Low
+ 0.10 mg. α. tocopheryl acetate
+ 0.25 mg. α. tocopheryl acetate
+ 0.75 mg. α. tocopheryl acetate

Graph 1

TABLE 1

Sexual physiology of males in the five groups.

GROUP	NO. OF RATS	ATTEMPTED MATINGS WITH OESTRUS FEMALES	INSTANCES OF PLUG OR SPERM	"REFUSED" MATINGS	FEMALES MATING THAT FAILED IMPLANTATION	NO. OF LITTERS SIRED
				%	%	
	(Fourth through Ninth Month)					
E-low	10	100	87	13	93	6
0.1 mg. α-tocopheryl acetate	10	100	83	17	52	40
0.25 mg. α-tocopheryl acetate	10	100	92	8	6	87
0.75 mg. α-tocopheryl acetate	10	100	91	9	2	89
(Natural food DietI) + lettuce	10	100	84	16	5	80
	(Tenth through Fifteenth Month)					
E-low	10	100	63	37	100	0
0.1 mg. α-tocopheryl acetate	10	100	63	37	87	7
0.25 mg. α-tocopheryl acetate	10	100	88	12	77	23
0.75 mg. α-tocopheryl acetate	10	98	92	6	11	78
Natural food (Diet I) + lettuce	9	99	82	9	12	69

TABLE 2

Age of male rats in the several groups of ten each at the first sterile mating (to 16 months).

GROUP	NUMBER STERILE	AGE IN MONTHS
E-Low	5	4
	9	4½
	10	5
0.1 mg. alpha tocopheryl acetate	1	4½
	5	5
	6	5½
	7	8
	8	11
	10	12
0.25 mg. alpha tocopheryl acetate	4	9½
	7	11
	8	16
0.75 mg. alpha tocopheryl acetate	None	16
Diet I + Lettuce	None	16

11 to 12 months. Uniformly sterile matings resulted in the final 4 months (tables 1 and 2). As contrasted with the vitamin E deficient state, the lowest intake of alpha tocopherol thus had some detectable effect. On autopsy at the seventeenth month the testicles presented the appearance of little bags of water, a characteristic of the vitamin E low organ. The weights of these organs were appreciably greater than for the E low group (table 3). The histological picture of "complete degeneration" of the testicular epithelium was uniformly encountered.[4]

TABLE 3

Summary of organ weights (males — age 17 months).

	E-LOW DIET	α TOCOPHERYL ACETATE			DIET I AND LETTUCE
		0.10 mg.	0.25 mg.	0.75 mg.	
No. of rats	4	5	5	5	5
Body weight (gm.)	409 344–476	522 466–594	559 486–670	577 488–736	545 405–664
Testes (gm.)	1.08 0.97–1.19	1.47 1.20–1.77	2.00 1.32–2.96	3.70 3.22–4.54	3.58 3.15–4.22
Seminal vesicles (gm.)	1.88 1.51–2.21	1.90 1.00–2.54	1.79 1.13–2.22	2.01 1.34–2.90	1.43 0.81–1.77
Prostate (gm.)	0.97 0.70–1.21	1.01 0.78–1.19	1.08 0.86–1.45	1.23 0.85–1.70	1.16 0.76–1.35
Cowper's glands (mg.)	108 90–142	116 87–134	114 85–129	118 114–123	135 101–217
Adrenals (mg.)	54 48–61	54 48–65	53 47–60	52 44–61	45 25–66
Thyroids (mg.)	40 29–53	47 39–59	47 32–69	38 30–47	77 52–99
Liver (gm.)	18.60 15.16–22.16	18.04 16.15–21.41	17.81 15.10–21.26	17.76 14.48–23.46	15.88 11.20–20.36
Kidneys (gm.)	3.30 2.86–4.13	3.36 3.20–3.58	3.44 2.91–4.14	3.55 3.30–3.85	2.97 2.42–3.55
Heart (gm.)	1.59 1.35–1.84	1.56 1.27–1.85	1.56 1.33–1.86	1.49 1.36–1.63	1.49 1.16–1.78

[4] It will be noted that the accessory organs of reproduction did not show the hypertrophy reported for younger animals (Evans, Emerson and Emerson, '39).

The performance of the rats receiving 0.25 mg. of alpha tocopheryl acetate was, in general, satisfactory during the first 9 months of life. Sterility had supervened in a majority of cases by the end of the first year as is seen from tables 1 and 2.[5] The testes of the animals in this group were subnormal in weight and usually showed marked degeneration.[6]

The rats on the high level of the tocopherol (0.75 mg.) were slightly superior in reproductive behavior to their stock ration brothers during the period studied.

The results of these tests indicate that the daily requirement for alpha tocopheryl acetate, as a supplement to the vitamin E low diet (Diet 427) is more than 0.25 mg. per day and the actual figure lies between this value and 0.75 mg.

The importance of long term experiments is seen from the fact that our results indicate that the inadequacy of 0.25 mg. daily of tocopherol may in some instances not disclose itself until 9½ months. The ages of the rats in the several groups at the onset of sterility are summarized in table 2.

The requirement of the female rat for alpha tocopheryl acetate

The female rats on the E-low ration showed a plateau in the weight curve at an average age of 4 months. The rats receiving the three levels of the tocopherol grew as well as did their sisters reared on the natural food ration (graph 2). The animals receiving 0.75 mg. were slightly superior (although not significantly so) to those receiving 0.1 and 0.25 mg.

Each individual female rat employed in the reproductive studies was bred with normal males until three litters were secured. The first breeding was initiated at the first proestrus after 90 days. All fifteen of the vitamin E deprived females underwent typical resorption gestations. The second

[5] Table 2 shows that two rats in the group retained fertility until the sixteenth month.

[6] Curious and unaccountable variations were noted as regards the exact histological condition of the testes in the 0.25-mg. group, for in some instances there were a few normal testicular tubules surrounded by degenerated tubules (fig. 4). At this age, as is perhaps unnecessary to state, complete degeneration of the tubules was found in the E-low and 0.10-mg. groups.

TABLE 4

Summary of organ weights — (females, age 19 months).

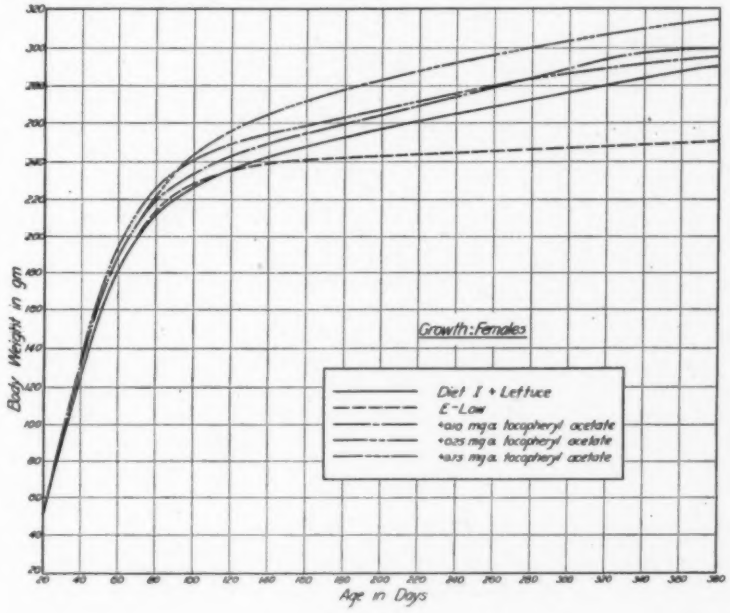
GROUP	NO. OF RATS	BODY WEIGHT	UTERUS & OVIDUCTS	OVARIES	ADRENALS	THYROIDS	LIVER	KIDNEYS	HEART
		gm.	*gm.*	*mg.*	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*
E-Low [1]	3	253	1.41	44	83	30	9.74	2.21	1.08
		214–295	0.65–2.61	33–59	70–104	17–43	8.60–11.91	1.97–2.63	. . .
0.1 mg.[1] α-tocopheryl acetate	5	326	0.93	59	71	31	11.62	2.09	1.00
		275–398	0.76–1.06	48–76	51–91	20–38	8.94–14.07	1.83–2.38	0.86–1.14
0.25 mg.[1] α-tocopheryl acetat	5	346	0.97	69	81	35	11.40	2.48	1.05
		293–434	0.69–1.18	33–103	60–101	28–52	9.40–14.06	1.87–2.82	0.90–1.13
0.75 mg.[1] α-tocopheryl acetat	5	369	1.05	56	78	35	12.48	2.43	1.14
		345–423	0.55–151	27–76	68–91	13–45	11.17–13.78	2.16–2.75	1.05–1.30
Diet I + Lettuce [1]	5	320	1.29	59	66	36	12.07	2.19	1.09
		265–388	0.75–2.05	32–93	49–109	21–50	9.26–17.24	1.74–3.45	0.82–1.41
E-Low [2]	5	266	1.31	35	76	27	10.21	2.12	1.17
		212–339	0.83–1.53	26–59	60–92	17–37	8.50–12.69	1.62–2.67	0.98–1.44
0.10 mg.[2] α-tocopheryl acetat	5	359	1.09	52	66	32	11.65	2.38	1.06
		331–401	0.67–1.24	30–85	48–89	26–46	9.77–13.75	2.04–2.68	0.93–1.20
0.25 mg.[2] α-tocopheryl acetat	5	359	0.85	58	71	35	11.07	2.46	1.13
		279–419	0.60–1.27	28–114	55–91	27–42	9.84–11.64	2.30–2.64	1.07–1.19
0.75 mg.[2] α-tocopheryl acetat	5	355	0.79	62	63	22	11.26	2.43	1.15
		331–388	0.56–1.11	35–100	59–75	16–29	10.00–12.26	2.31–2.50	1.12–1.19
Diet I + Lettuce [2]	5	307	0.61	55	59	39	9.85	2.08	1.01
		271–335	0.47–0.76	30–116	45–73	27–45	8.42–11.30	1.80–2.41	0.91–1.14

[1] Virgin females

[2] Breeding females

breeding during the seventh and eighth months, and the third breeding in the tenth and eleventh months yielded similar results.

The animals showing the implantation sign littered on all levels of the tocopherol (table 5). The average size of the litters and the average weight of the young were essentially the same for all treated groups as for the stock ration.



Graph 2

It is of interest to note that the typical dark brown discoloration of the uterus reported by Martin and Moore ('36) was observed in all animals in the E low group and in those receiving 0.1 and 0.25 mg. of the tocopherol (autopsied at 19 months), but not in those receiving 0.75 mg.

As was the case with the males, the striated musculature was found to be normal at autopsy on the nineteenth month in all groups receiving tocopherol, whereas the females in the

TABLE 5

Summary of reproductive behavior of females and condition of young.

LITTER	GROUP	NUMBER OF RATS	NUMBER OF LITTERS	TOTAL NUMBER OF LIVING YOUNG	AVERAGE WEIGHT AT BIRTH	% SURVIVING ON DAY 21	AVERAGE WEIGHT ON DAY 21	% SURVIVING ON DAY 30	AVERAGE WEIGHT ON DAY 30	AVERAGE DAY PARALYSIS NOTED	% YOUNG PARALYSED	% YOUNG RECOVERED ON DAY 30
					gm.		*gm.*		*gm.*			
First	E-Low	15	0									
	0.1 mg.	15	15	133	5.7	75	46	43	62	Day 20	100	23
	0.25 mg.	15	15	123	5.8	70	51	67	85	Day 20	24	82
	0.75 mg.	15	14	115	5.8	84	53	84	92	None	None	
	Diet I	14	14	130	5.9	89	47	89	83	None	None	
Second	E-Low	13	0									
	0.1 mg.	14	12	110	5.9	73	46	48	53	Day 20	100	10
	0.25 mg.	14	14	122	5.8	82	54	80	85	Day 21	54	58
	0.75 mg.	14	13	114	6.2	93	54	92	88	None	None	
	Diet I	14	11	87	6.3	89	48	93	88	None	None	
Third	E-Low	8	0									
	0.1 mg.	12	9	50	5.9	39	43	3	45	Day 20	100	None
	0.25 mg.	14	10	64	5.4	75	47	48	55	Day 21	100	None
	0.75 mg.	14	10	82	5.6	82	52	80	88	Day 23	30	100
	Diet I	14	8	58	5.9	75	56	73	88	None	None	

E low group showed motor impairment and histological lesions in the striated musculature.

The requirement of suckling young for alpha tocopherol

The mothers in the several groups were allowed to suckle six young. At the onset of lactation these mothers and the young in these groups were indistinguishable. All were vigorous and of normal size. As regards the first litters from mothers receiving 0.1 mg. tocopherol, the suckling young appeared normal until the twentieth day when the characteristic muscular dystrophy known in E deficiency appeared. The young from mothers receiving this level of tocopherol were uniformly dystrophic.[7] Approximately one half of the young

[7] This particular intake of alpha tocopherol seemed ideal for the production of this clinical picture. The condition has previously been produced experimentally by allowing females exhibiting first litter fertility to suckle their young or by the administration of sufficient alpha tocopherol or another source of vitamin E to a pregnant female of previously established E-avitaminosis. Under such experimental conditions all young are not dystrophic.

survived 30 days. Twenty-three per cent of the animals that had been dystrophic prior to 30 days had recovered by that time. The weights of such recovered animals were markedly subnormal despite the fact that the food was accessible, i.e., placed in a low dish on the floor of the cage.

The 0.25-mg. level of alpha tocopheryl acetate did not afford complete protection against the dystrophy as 24% of the young from these mothers were paralyzed; however, the degree of impairment was greatly reduced at this level and 82% showed complete clinical recovery by day 30. The weights for the young in this group were normal.

The young reared by mothers on the highest level of tocopherol (0.75 mg.) were normal in all respects and surpassed their stock ration cousins in weight and general well-being.

A second breeding resulted in almost identical findings; note should be taken however, of an increased incidence of dystrophy in the 0.25-mg. group. When discussing the males, emphasis was given the fact that inadequacy of the lower levels of tocopherol appeared relatively late. The same phenomenon was seen as regards muscular dystrophy in the suckling young.

As regards the third breeding, on the lowest (0.10 mg.) level of the tocopherol only 3% survived 30 days and 100% of the young in the 0.25-mg. group were paralyzed; all animals living at 30 days showed some evidence of dystrophy. Some slight muscular impairment was noted in the young of mothers on the highest level (0.75 mg.) of alpha tocopheryl acetate; however, all had recovered by the thirtieth day.

DISCUSSION

These studies indicate that with the basal diet here employed the daily requirement of the rat for alpha tocopheryl acetate for normal growth and normality of the striated musculature is approximately 0.1 mg., although the actual value may be somewhat less. The daily requirement for normal reproduction in the male is somewhat below 0.75 mg. For the prevention of muscular dystrophy in suckling young born

after the tenth month of life, more than 0.75 mg. of tocopherol daily is required (table 5, third litters). These figures are many times higher than the optimal level suggested by Mason, but it must be pointed out that Mason's experiments were of short duration and that his animals were sacrificed at the age at which our breeding tests were initiated. The basal diets were not identical. Mason did not study the tocopherol requirement during lactation for the prevention of muscular dystrophy in the suckling young.

The quantity of vitamin E received by the mother does not materially affect either the quantity or quality of milk produced save in the one respect as to its E content, for the young prior to the onset of paralysis are usually normal in size.

SUMMARY

1. Growth and reproduction were studied for 16 months in male and female rats maintained from weaning on a vitamin E low diet (Diet 427), and supplemented with three levels of alpha tocopheryl acetate; the levels were 0.10 mg., 0.25 mg., and 0.75 mg. six times weekly.

2. The tocopherol requirement for normality of the striated musculature and for growth in the case of both sexes is less than 0.10 mg. daily.

3. As regards the males, the 0.10-mg. level was inadequate for the preservation of fertility beyond the fifth month. The 0.25-mg. level was adequate for normal reproduction during approximately the first 9 months of life, but proved inadequate for the subsequent maintenance of fertility. The highest level of tocopherol (0.75 mg. daily) maintained normal testes and fertility during the entire experimental period of 16 months.

4. As regards the females, those receiving 0.10 mg. daily were able to give birth to living young even in their third gestation which was completed between the eleventh and twelfth months of age. By the eighteenth month orange-brown pigmentation of the uterus supervened in all animals receiving 0.10 mg. and 0.25 mg. daily dosage but was absent in the group receiving 0.75 mg.

5. As regards the suckling young, in contrast to the adult female, all those born from mothers receiving 0.10 mg. daily dosage exhibited muscular dystrophy with a high mortality rate in the third week of life. Those born from mothers receiving 0.25 mg. daily dosage exhibited a lessened incidence of dystrophy with a lowered mortality rate until the third breeding when all young were paralyzed and died as was the case in litters from mothers receiving 0.10 mg. All young from mothers receiving 0.75 mg. daily dosage were normal in the first two breedings, but in the third gestation there was a slight incidence of dystrophy with complete recovery by the thirtieth day of life.

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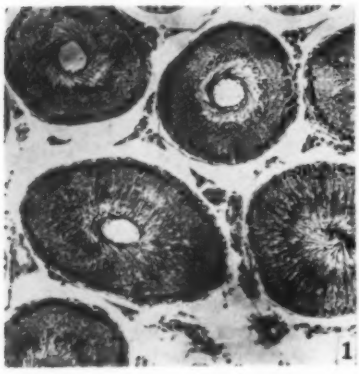

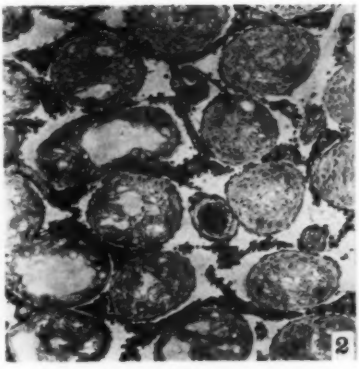

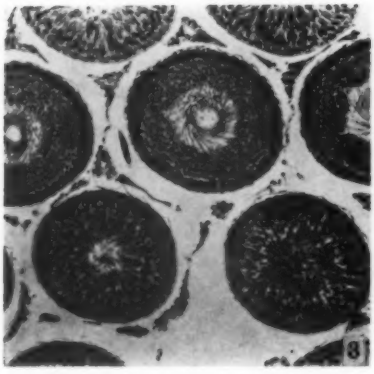

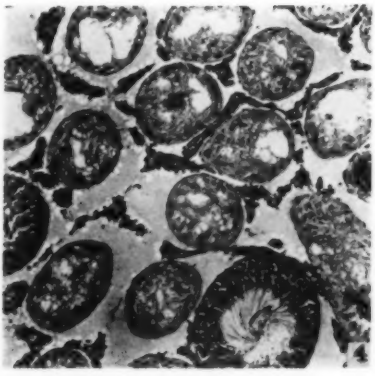

1 Typical area from cross section of the testis of rat 17 months old, reared and maintained on natural stock diet (Diet I and lettuce). × 135.

2 Typical area from cross section of testis of 17-month-old rat, reared and maintained on vitamin E-low diet (Diet 427). Complete degeneration of the seminiferous epithelium is shown. This rat was sterile after 4½ months of age. × 135.

3 Typical area from cross section of testis of 17-month-old rat reared and maintained on vitamin E-low diet but supplemented with 0.75 mg. α-tocopheryl acetate six times weekly. × 135.

4 Cross section of testis of rat 21 months old, reared and maintained on vitamin E-low diet supplemented with 0.25 mg. α-tocopheryl acetate six times weekly. × 135.

THE INFLUENCE OF NUTRITION DURING PREGNANCY UPON THE CONDITION OF THE INFANT AT BIRTH

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TWO FIGURES

Nutrition has not occupied a place of major importance as a part of prenatal care. The obstetrician's primary interest is not so much the health and development of the unborn infant as the health of the mother during pregnancy. Interest in diet during the gestation period, except in so far as it relates to excessive weight gain of the mother, has been relatively slow to develop, because the medical profession has not been convinced that diet during pregnancy occupies a place of great importance in regard to either the mother or her offspring. Heredity, nutrition, activity and disease are the principal factors which determine the course of physical growth and development. The relative importance of each has not been established, but it is becoming increasingly clear that "from conception to death, health is primarily dependent upon food and nutrition" (Macy, '42). It is also recognized (Adair, '32) that although in the earliest part of pregnancy the ovum carries some nutrient material within itself, it is largely dependent upon the mother for the material from which it is to grow and develop.

The need for better nutrition during the period of pregnancy has been repeatedly emphasized (Garry and Stiven, '36; Dieckmann and Swanson, '39; Burke, '41). While no attempt will be made here to review the literature completely, by way of historical background, attention is called to a few of the many illustrations of fetal damage resulting from prenatal dietary deficiency of various types which are to be found in the records of animal experimentation (Hart, Steenbock and Humphrey, '20; Smith, '17; Hale, '37; Warkany and Nelson, '42). Interesting evidence of damage to the human fetus also, due to inadequate maternal nutrition during pregnancy, has been reported (Maxwell, Pi, Lin and Kuo, '39; Toverud, '38; Burke, '40; Editorial, '32; Teel, Burke and Draper, '38). The metabolism studies of Macy and her coworkers ('42) and of Coons, Schiefelbusch, Marshall and Coons ('35), carried out on women during the reproductive cycle, contributed to our knowledge of the nutritional requirements of women in this period. The recent works of Ebbs, Tisdall and Scott ('41) evaluates the diet of the mother during pregnancy in relation to her condition during the course of pregnancy, labor, delivery, and the postpartum period, as well as to the condition of the infant at birth and within the early months of life. Recent English studies (Interim Report, '42) report the effect of improved nutrition of expectant mothers on maternal and infant morbidity and mortality by the use of concentrates.

GROUP STUDIED AND METHODS

The study of the influence of diet during pregnancy upon fetal growth and development, as well as upon the course of pregnancy, labor, delivery, and the puerperium, is a part of the research program on the growth and development of the well child, undertaken by the Department of Child Hygiene of the Harvard School of Public Health. The work was begun in 1930, and the program enlarged to include the establishment of a Center for Research in Child Health and Development in

1932.[1] A detailed description of the work at the Center is given in an early publication (Stuart and Staff, '39).

Three-hundred and twenty-four infants have been enrolled at the Center. In this paper observations on 216 women and their infants are presented. In each of the 216 cases the mother has been enrolled at the Center for the first time, and the resulting infant is the oldest sibling of that family in the study. Pregnancies resulting in younger siblings and in twins have been omitted but will be presented in a later paper. This main group of mothers and their infants represents a sampling of cases uninfluenced previous to the pregnancy under study.

The women were selected from the prenatal clinics of the Boston Lying-in Hospital and were cared for in the early years of the study by the staff of that hospital. Since 1935 they have been under the care of obstetricians attached to the staff of the Center, who have also been members of the hospital staff. These women were seen at least monthly through the seventh month of pregnancy, every 2 weeks during the eighth month, and then weekly, unless it became necessary to see them more frequently because of complications.

The pediatric ratings describing the condition of each infant at birth and within the first 2 weeks of life are based upon the obstetrician's evaluation of the infant's condition at birth, together with a routine physical examination of each infant by a pediatrician from the staff of the Center within 48 hours of birth (the majority within 24 hours of birth), and another routine physical examination by a staff pediatrician before discharge from the hospital, usually on the fourteenth day of life. Careful notes were made of the progress of each infant while in the nursery, and all infants whose neonatal course was not entirely satisfactory were visited frequently during that period by one of the Center's pediatricians.

[1] The work of the Center was made possible in part under a grant from the General Education Board of the Rockefeller Foundation. The Nutrition studies have been supported in part by a grant from the Forsyth Dental Infirmary for Children, Boston.

The nutrition data are based upon detailed dietary histories obtained at the time of the mother's first visit to the prenatal clinic and at as many subsequent visits as the individual case required, at least once each trimester. These nutrition histories include a complete record of the food intake for the 24-hour period preceding the clinic visit, data about the woman's normal food habits and any changes which have been made in these during the course of pregnancy. In addition, at regular intervals during the course of pregnancy each patient kept a consecutive 3-day record of food consumed. Information in regard to food likes and dislikes, amounts of various foods purchased, amount of money spent for food in relation to total weekly income and number of persons in the family, and other related data, have been recorded. The average consumption of important foods, such as milk, eggs, meat, fish, poultry, liver, whole grain cereals and breads, vegetables, fruit, etc., have been carefully checked. An investigation was also made of the amount of highly refined carbohydrate and fat-rich foods eaten, in an attempt to determine to what extent foods of these types were replacing foods rich in proteins, minerals, and vitamins. Such a method of cross-checking important data has made a valuable contribution in greater accuracy of the final dietary ratings. The forms used in taking these nutrition histories, and further details as to the method, are given in a monograph (Stuart and Staff, '39).

The method used in evaluating these diets is also described in detail in another publication (Burke and Stuart, '38). In evaluating these diets for the last two trimesters of pregnancy, comparison has been made with an arbitrary set of standards (table 1) selected as "optimal," which approximate the values later recommended by the Food and Nutrition Board of the National Research Council.

No nutrient was called "excellent" unless it met or exceeded this "optimal" standard. Each nutritional essential was rated "excellent", "good", "fair", "poor", or "very poor", according to the range within which it appeared to fall. If the variations in the intake of any nutrient made the exact rating

TABLE 1

Nutritional standards used in rating dietary intakes during pregnancy (4th through 9th months)

NUTRITIONAL ESSENTIALS	EXCELLENT	GOOD	FAIR	POOR	VERY POOR
Protein, gm.	85 or more	70–84	55–69	45–54	Under 45
Calcium, gm.	1.5	Under 1.5–1.0	Under 1.0–.75	Under .75–.6	Under .6
Phosphorus, gm.	2.0	The same rating was given phosphorus in each case as was assigned to protein.			
Iron, mg.	20	Under 20–16	Under 16–12	Under 12–10	Under 10
Vitamin A, I.U.	8000	Under 8000 to 6000	Under 6000 to 4000	Under 4000 to 2000	Under 2000
Thiamine, mg.	2.0	Under 2.0–1.0	Under 1.0 to above .5	.5–.3	Under .3
Riboflavin, mg.	2.5	Under 2.5–2.0	Under 2.0–1.5	Under 1.5–1.2	Under 1.2
Niacin, mg.	18	No attempt was made to rate niacin.			
Ascorbic acid, mg.	100	Under 100–75	Under 75–50	Under 50–35	Under 35
Vitamin D, I.U.	400–800	These were used merely as "excellent" with added D and "poor" without added D.			

of the average intake doubtful, a rating has been assigned to indicate this, for example, "fair to poor". Therefore, each dietary rating is an average estimate and falls approximately within a range represented by the terminology "excellent", "good", "fair", etc., as shown in table 1. A daily caloric intake above 2800 has been termed "excessive" and below 2200 Calories "inadequate" in all cases. Judgment in regard to the individual case has to a large extent determined the rating assigned, if the caloric intake was between these two extremes. A closer analysis of data obtained by the nutrition history method is unwarranted, and more exact calculation of diets is justified only in the case of weighed samples.

The mean general dietary rating used as representative of each diet during the major portion of pregnancy was obtained by averaging numerical values given to the ratings assigned to the nutritional essentials: "excellent" = 4, "good" = 3, "fair" = 2, "poor" = 1, and "very poor" = 0. This method of obtaining the mean rating assigned to the average dietary intake of each woman during pregnancy has resulted in more accurate evaluations when used in comparative case studies, as the relative relationship of each dietary rating to every other is exact. This has greatly facilitated the statistical handling of the nutritional data.

Of the 216 cases studied, only 14% of the women consumed diets during pregnancy (fourth through ninth month) which could be considered "excellent" or "good" according to the nutritional standards given in table 1. The diets consumed by 17% of the women were "fair to good", 29% had "fair" diets, 23% "fair to poor" diets, and 17% had diets for this period which were "poor to very poor". This means that at least 40% of the women in this group were definitely malnourished, according to these dietary standards, during a period when the fetus undergoes very rapid growth and development, and that another 29% had only mediocre diets for this period.

The parents of these infants are largely of North European stock, as is shown in table 2, and represent, from an economic standpoint, the average "middle class" American family.

The economic depression of the early nineteen-thirties intervened to move a number of families to a lower economic level. A few cases from the upper economic brackets are included, usually individuals close to the study staff who provided an opportunity for the collection of unusually reliable data. Table 3 shows the relationship of income to number of dependents within each family during the pregnancy under study.

The ages of the parents at the time of the birth of these infants are given in table 4.

TABLE 2

Racial background of parents.

RACIAL BACKGROUND	NUMBER OF CASES	PER CENT
Irish	46	21.3
"American" or "Canadian"	18	8.3
French-Canadian, French, Scotch, English, Irish	23	10.6
Scotch, English, Irish	56	25.9
Scandinavian, Finnish, German, Dutch, American, etc.	48	22.2
TOTAL OF COMBINED NORTH EUROPEAN STOCK	191	88.3
Italian or Part Italian	9	4.2
Jewish or Part Jewish	9	4.2
Others (Albanian, Polish, Syrian, etc.)	7	3.3
TOTAL OTHER THAN NORTH EUROPEAN STOCK	25	11.7
Total	216	100.0

The medical data concerning the mothers, together with the findings in regard to the influence of diet during pregnancy upon the course of pregnancy, labor, delivery and the postpartum period, are given in a recent publication (Burke, Beal, Kirkwood, and Stuart, 1943).

The pregnancies of these women terminated in an almost equal number of male and female infants, 107 male and 108 female infants, and one stillborn infant whose sex was undetermined. There were 200 full term, 9 premature,[2] 5 stillborn infants, and 2 infants who died within a few hours of birth.

[2] Three of these infants were included because one specialist in the group was particularly interested in the study of premature infants.

TABLE 3

Relation of income to number of dependents in family during prenatal period.

INCOME PER WEEK	SIZE OF FAMILY		NUMBER OF CASES	PER CENT
$19.00 or less	2 Adults	0 Children 17	21	9.7
	2 Adults	1 Child 3		
	2 Adults	2 Children 1		
$20.00–$24.00	2 Adults	0 Children 22	30	13.9
	2 Adults	1 Child 6		
	2 Adults	2 Children 1		
	2 Adults	6 Children 1		
$25.00–$29.00	2 Adults	0 Children 40	52	24.1
	2 Adults	1 Child 9		
	2 Adults	2 Children 3		
$30.00–$39.00	2 Adults	0 Children 58	76	35.2
	2 Adults	1 Child 15		
	2 Adults	2 Children 2		
	2 Adults	3 Children 1		
$40.00–$49.00	2 Adults	0 Children 18	23	10.6
	2 Adults	1 Child 3		
	2 Adults	2 Children 2		
$50.00 or more	2 Adults	0 Children 6	12	5.6
	2 Adults	1 Child 4		
	2 Adults	2 Children 2		
No data	2 Adults	0 Children 2	2	0.9
Total number of families			216	100.0

TABLE 4

Age of parents at infants' birth.

AGE DISTRIBUTION	MOTHERS		FATHERS	
	No. cases	%	*No. cases*	%
20 yrs. or under	24	11.1	5	2.3
21–25 yrs.	79	36.6	63	29.1
26–30 yrs.	70	32.4	76	35.2
31–35 yrs.	33	15.3	52	24.1
36 yrs. or over	10	4.6	20	9.3
Total	216	100.0	216	100.0

RESULTS AND DISCUSSION

In considering any possible effects of nutrition during pregnancy on infant mortality and morbidity, the diet of the mother during pregnancy was studied in relation to the condition of the infant at birth and during the first 2 weeks of life.[3] All of the 216 infants against whom the examining pediatricians had found no physical count of any kind, either at birth or within the first 2 weeks of life, were selected as the "superior" infants of the group. There are twenty-three in this group. The birth weights and lengths of these infants, together with the mean general ratings assigned to the mothers' diets during pregnancy (fourth through ninth months) are given in table 5.

In contrast, all infants at the opposite extreme of the group in pediatric rating were next considered. The "poorest" infants included all who were stillborn, died within a few hours or days of life, had marked congenital malformations at birth, were premature (under 5 lbs. at birth), or were "functionally immature." "Functionally immature" means that the physical development or reactions of the infant were considered below normal by the pediatrician, and does not apply to weight and length alone. There were thirty-three infants in the poorest group, and the data concerning them are presented in table 6.

The remaining infants, of whom there are 160, were subdivided into two groups. All infants against whom the pediatric rating showed only one or two minor physical counts were considered in "good" physical condition. There are eighty-four infants in this classification. All infants not in one of the three already defined classifications were placed in one group termed "fair" (some of these were considered to be in fair to poor physical condition, others were definitely in a fair to good classification according to the pediatric findings). There are seventy-six "fair" infants. In interpreting these

[3] It should be remembered in studying the relationships obtained that the ratings used in each field (pediatric, obstetric, nutritional, anthropometric, etc.) have been made by the specialist in charge of each field of endeavor, and the results assembled to determine possible associations of statistical significance.

groupings, it should be remembered that those infants termed "good" are relatively near the superior infants in physical condition, while the group called "fair" represent a much wider range. The relationship existing between the physical condition of the infant and the mother's diet during pregnancy

TABLE 5

Mean ratings of prenatal diets, birth weights, and birth lengths of "superior infants".[1]

CASE NO.	CONDITION OF INFANT	WEIGHT		LENGTH	PRENATAL DIET MEAN RATING
		lbs.	*ozs.*	*cm.*	
5	All infants are "Superior"	7	0	50.5	Fair
16		6	12	47.5	Excellent
20		7	0	49.2	Good
24		8	2	52.1	Fair to poor
37		10	3	53.1	Excellent
43		8	14	53.3	Fair to good
49		8	14	51.6	Good
52		9	2	51.8	Good
91		8	9	51.4	Excellent
133		7	11	51.0	Good
142		8	5	50.8	Good (to fair)
154		6	10	50.3	Poor (to very poor)
189		11	7	53.5	Good
192		8	8	50.7	Fair
198		6	15	47.6 (7 days)	Fair
207		7	4	50.6	Fair
210		7	15	50.6	Fair
220		7	13	51.2	Good to excellent
240		7	14	51.2	Fair
248		7	12	51.4	Fair
265		7	11	51.4 (5 days)	Good
290		8	8	49.9	Good
323		8	5	52.1 (5 days)	Excellent

[1] All infants against whom there was no physical count of any kind recorded either at birth or within the first 2 weeks of life.

is shown graphically for the entire 216 cases in figure 1 (cases selected on the basis of pediatric ratings).

Similar correlations are found when the cases are selected on the basis of the rating assigned to the diet of the mother during pregnancy. This relationship is shown graphically in figure 2 (cases selected on the basis of prenatal dietary rat-

TABLE 6

Mean ratings of prenatal diets, birth weights and birth lengths of "poorest" infants.[1]

CASE NO.	CONDITION OF INFANT	WEIGHT		LENGTH	PRENATAL DIET MEAN RATING
		lbs.	*ozs.*	*cm.*	
17	Stillborn	5	12	(54.6) [2]	Very poor
36	Stillborn	6	15	(50.8) [2]	Very poor
48	Stillborn. (Macerated)	4	12	.....	Very poor
110	Stillborn	7	8	.....	Poor (to very poor)
237	Stillborn. Macerated. Multiple congenital anomalies.	Del. 3 mos. premature		.....	Very poor
199	Died at birth. Erythroblastosis. Congenital urethral stenosis.	8	15	(53.3) [2]	Poor to very poor
205	Died in 2 hours. Multiple congenital anomalies.	6	12	48.5	Fair
8	Died 3rd day (bronchitis & broncho-pneumonia)	6 (2 days)	1	(52.1) [2]	Poor (to very poor)
235	Died at 5 mos. Congenital malformation heart	3	4	.. .	Very poor
81	Congenital heart	8	11	52.6	Excellent
127	Congenital heart	7	0	49.4	Poor to very poor
108	Mild erythroblastosis	7	2	50.6	Very poor 7 mo. then good to excellent
116	Bifid uvula	8	9	52.3	Fair (to poor)
58	Congenital mobile funnel chest	8	14	51.6	Fair
120	Arrested hydrocephalus Premature	3	12	40.6 (14 days)	Poor to very poor
231	Cleft palate. (Feeble-minded)	8	0	48.9	Poor to very poor
191	Inguinal hernia. Premature	3	6	44.5 (26 days)	Fair
122	Congenital cataracts. Premature (mentally retarded at 1 yr.)	3	5	41.9 (14 days)	Poor to very poor
158	Premature	3	14	43.2 (30 days)	Poor to very poor
204	Premature	4	11	47.0 (11 days)	Poor to very poor
206	Premature	3	12	44.5 (30 days)	Poor to very poor
211	Premature	5	0	45.7 (5 days)	Poor to very poor
281	Premature	4	13	45.7 (14 days)	Poor to very poor
69	Functionally immature	5	14	48.9 (13 days)	Very poor
42	Functionally immature	6	0	46.6	Poor to very poor
67	Functionally immature	5	6	47.4	Fair
76	Functionally immature	5	4	48.5	Poor to very poor
93	Functionally immature	6	6	50.6	(Fair to) poor
109	Functionally immature	6	4	48.4	Poor (to very poor)
125	Functionally immature	5	13	47.0 (6 days)	Poor (to very poor)
196	Functionally immature	5	8	45.6	Poor to very poor
213	Functionally immature	6	12	48.2	Fair
238	Functionally immature	6	12	48.0	Fair (†not utilized)

[1] All infants who were stillborn, died within a few hours or days of life, had a marked congenital malformation at birth, were premature (under 5 lbs. at birth) or were "functionally immature."

[2] Not measured by our staff; taken from hospital records.

ings). Table 7 gives the birth weights and lengths of the infants grouped according to the pediatric rating and according to the rating assigned to the mother's diet during pregnancy.

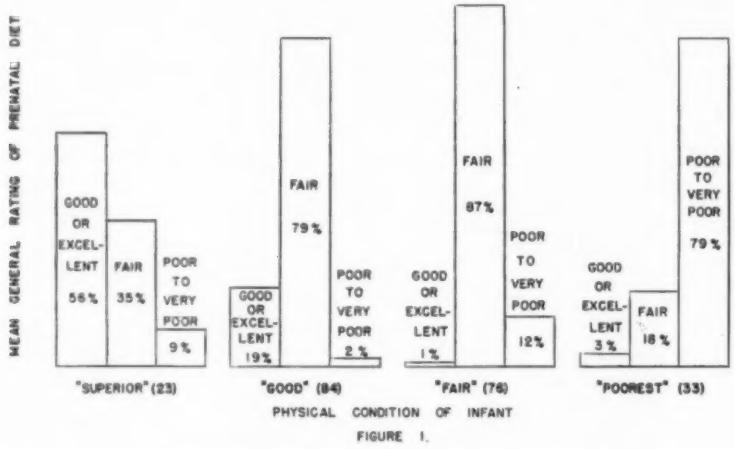

Fig. 1 Relationship of the condition of infant at birth and within first 2 weeks of life to mother's diet during pregnancy. (Cases selected on the basis of pediatric ratings of infants, 216 cases).

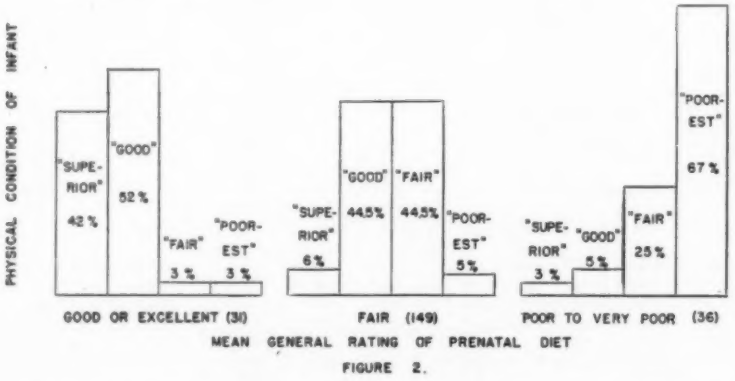

Fig. 2 Relationship of prenatal nutrition to the physical condition of the infant at birth and within first 2 weeks of life. (Cases selected on the basis of mean rating assigned to mothers' diets during pregnancy, 216 cases).

TABLE 7

Birth weights and lengths of infants grouped according to pediatric rating and according to prenatal dietary rating.

	PEDIATRIC RATING				PRENATAL DIETARY RATING		
	"Superior"	"Good"	"Fair"	"Poorest"	Excellent or Good	Fair	Poor to Very Poor
Birth weight lbs., oz.							
Average	8–2	7–12	7–2	5–15	8–8	7–7	5–13
Range	6–10 to 11–7	5–8 to 10–12	5–4 to 9–3	3–4 to 8–15	6–12 to 11–7	3–6 to 9–3	3–4 to 8–15
Birth length cm.							
Average	50.8	50.6	49.5	47.2	51.8	50.0	47.2
Range	47.0 to 53.3	46.2 to 54.4	45.0 to 54.4	40.6 to 52.1	46.9 to 54.6	45.0 to 54.4	40.6 to 52.7

CONCLUSIONS

1. A statistically significant relationship has been shown between the diet of the mother during pregnancy and the condition of her infant at birth and within the first 2 weeks of life.

2. If the diet of the mother during pregnancy is poor to very poor, she will undoubtedly have an infant whose physical condition will be poor. In the 216 cases considered in this study, all stillborn infants, all infants who died within a few days of birth except one, most infants who had marked congenital defects, all premature, and all functionally immature infants were born to mothers whose diets during pregnancy were very inadequate.

3. If the mother's diet during pregnancy is good or excellent, her infant will in all probability be in good or excellent physical condition. It may, however, happen rarely that a mother whose diet during pregnancy is good or excellent will give birth to an infant in poor physical condition (1 out of 216 cases in this study).

ACKNOWLEDGMENT

The authors are indebted to Miss Jane Worcester of the Department of Vital Statistics for statistical assistance in the handling of the material included in this paper, to Mrs. Vernette Vickers Harding of the Center for Research in Child Health and Development, Harvard School of Public Health, for valuable assistance in the assembling of the data, and to Dr. F. J. Stare, Division of Nutrition, School of Medicine and School of Public Health, Harvard University, for assistance in the preparation of the paper.

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EFFECTS OF CECECTOMY, SUCCINYLSULFATHIAZOLE, AND p-AMINOBENZOIC ACID ON VITAMIN K SYNTHESIS IN THE INTESTINAL TRACT OF RATS[1]

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The synthesis of certain vitamins in the intestinal tracts of man and animals has been recognized for many years (Anonymous, '43). Guerrant, Dutcher and Tomey ('35), Griffith ('35), Tange ('39), and others, reported the effect of the type of carbohydrate in the diet in relation to intestinal synthesis of B vitamins. Nielsen, Shull and Peterson ('42) found that intestinal synthesis of biotin varied with different rations and was particularly low when the diet contained inadequate amounts of riboflavin. However, progress in the study of vitamin synthesis by intestinal microorganisms was greatly limited until certain sulfonamides with satisfactory bacteriostatic effects were introduced (Black, McKibbin and Elvehjem, '41; Mackenzie, Mackenzie and McCollum, '41; Welch, '42; Poth and Ross, '43). The availability of succinylsulfathiazole (sulfasuxidine), which seems in certain cases to be more suitable than sulfanilylguanidine (sulfaguanidine) (Welch, '42), has extended the possibilities for investigation along these lines.

The antagonizing effect of p-aminobenzoic acid on the inhibition of growth caused by sulfaguanidine was demonstrated

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[2] Some of the data reported in this paper were taken from a thesis to be submitted by Merle M. Krider in partial fulfillment of the requirements for the Master of Arts degree.

by Black, McKibbin and Elvehjem ('41), Mackenzie, Mackenzie and McCollum ('41) and others. Although it was first believed (Welch, '42) that p-aminobenzoic acid does not inhibit the bacteriostatic effect of sulfasuxidine, it has been recently reported that the compound does partially counteract the inhibiting effect of sulfasuxidine on growth (Welch and Wright, '43; Neumann, Krider and Day, '43). No reports have been made concerning the effect of p-aminobenzoic acid on the hypoprothrombinemia produced, under certain conditions, by feeding sulfasuxidine.

Investigation of the possible role of the cecum in vitamin synthesis was stimulated by Guerrant et al. ('35) who noted that the cecum was unusually large in rats fed carbohydrates which promote relatively abundant synthesis of certain B complex vitamins. Moreover, such ceca contained large numbers of live yeast cells. Griffith ('35) cecectomized over 200 weanling rats and determined the effect on growth when the diet was deficient in one or more groups of water soluble vitamins. Since coprophagy improved the condition of the operated animals it was concluded that vitamin synthesis occurs in other parts of the alimentary tract. Innes and Nicolaysen ('37) cecectomized about 100 young rats in order to study the effect of the cecum on phosphorus utilization from phytin. Taylor, Pennington and Thacker ('42) found that cecectomized rats fed a good stock diet were apparently unaffected but when the quality of the ration was impaired by dilution, largely with sucrose, the operated animals became markedly debilitated while the unoperated controls remained in good health. Their studies suggested that "where the diet supports the right intestinal flora, the cecum contributes to the rat's supply of several of the B vitamins." These investigations indicate the importance of the cecum in the synthesis of vitamins and the possible value of cecectomized animals in the study of such problems.

Several investigations have shown the synthesis of vitamin K by the intestinal flora of rats (Dam et al., '37; Greaves, '39) and of ruminants (McElroy and Goss, '40). Black, Overman,

Elvehjem and Link ('42) and Welch and Wright ('43) seem to be the only workers who have reported the effect of sulfonamides on this synthesis. Lockhart, Sherman and Harris ('42) noted extreme hypoprothrombinemia in rats fed dihydroxystearic acid. The deficiency syndrome could be cured or prevented by 2-methyl-1, 4-naphthoquinone.

The study to be reported here was carried out primarily to determine (1) whether the cecum is a significant source of vitamin K to the rat and (2) to what extent sulfasuxidine and p-aminobenzoic acid affect the synthesis of this vitamin in the intestinal tract.

EXPERIMENTAL

Young, piebald rats of the McCollum strain were used. In all experiments except those involving cecectomy the starting weight was 40 to 55 gm. Cecectomized animals weighed 60 to 80 gm. at the time of the operation. The animals were divided as equally as possible on the basis of litter membership, sex and weight. Although they were kept on screens, precaution was taken to keep the cages clean in order to reduce coprophagy to a mininum.

For prothrombin time determinations blood was obtained by cardiac puncture under ether anesthesia, except in the early part of the study when it was secured from the exposed inferior vena cava of the animals which were subsequently autopsied. In the latter cases only one sample, of course, was obtained from each animal. The blood, 1.8 ml., was drawn into a clean syringe containing 0.2 ml. of 0.1 M sodium oxalate and then carefully discharged into a clean 15 ml. centrifuge tube. The prothrombin time was determined on diluted plasma by a modification of Quick's method (Pohle and Stewart, '39). All the prothrombin times reported in this study are in terms of 12.5% plasma. In general, blood was taken for prothrombin determinations biweekly. Uniform procedures were applied throughout this study.

In analyzing the data on plasma prothrombin time all values above 50 sec. were interpreted as proof of definite hypopro-

thrombinemia. All values below 50 sec. were regarded as essentially normal. Overman et al. ('42), using approximately the same method as we have employed, found that the range of prothrombin time for normal rats on stock diets is about 36 to 45 sec. Our range of values for apparently normal rats is about 32 to 42 sec. Consequently it would seem that our criterion of hypoprothrombinemia is a conservative and valid one.

The experimental diets used are indicated in table 1. Vitamin mixture 1 contained thiamine, 10 mg.; pyridoxine, 10 mg.; riboflavin, 20 mg.; nicotinic acid, 200 mg.; calcium pantothenate, 200 mg.; inositol, 400 mg.; and glucose, 9.17 gm. Vitamin mixture 2 was the same except that it contained 200 mg. p-aminobenzoic acid and a correspondingly smaller amount of glucose. The dry vitamin-sugar mixture was made up in sufficient amounts to last several weeks and was kept in the refrigerator. Owing to the relatively large amount of sugar contained in the mixture the designated quantity of vitamins per kilogram of diet could be readily weighed on the scales used for the other components of the diets.

Salts 2 contained $NaHCO_3 \cdot H_2O$, 95; $CaHPO_4 \cdot 2H_3O$, 215; KCl, 152; $MgSO_4 \cdot 7H_2O$, 98; iron citrate, 10; $ZnSO_4 \cdot 7H_2O$, 2.5; $MnSO_4 \cdot 2H_2O$, 2.0; $CuSO_4 \cdot 5H_2O$, 1.0; and KI, 0.5.

When it became difficult to secure sucrose, because of the war rationing, glucose was substituted, as indicated in table 1.

Cecectomy

A paramedian incision is made and the skin flaps are separated along the fascial plane to expose the muscular layer of the abdominal wall. A median incision along the linea alba exposes the abdominal viscera with the least amount of hemorrhage. The cecum with its attachments to the terminal ileum and the proximal portion of the colon is lifted out of the peritoneal cavity through the opening in the abdominal wall and the laparotomy cloth is overlapped in order to protect the peritoneal cavity. The blood vessels supplying the cecum are doubly ligated and sectioned between ligatures and the ends

TABLE 1

Percentage composition of diets.

CONSTITUENTS	DIETS											
	1	2	3	4	5	6	7	8	9	10	11	12
Sucrose	70.5	70.5	70.5	70.5	69.6	70.6	69.6	70.6				
Glucose (Cerelose)									70.0	70.0	66.0	65.0
Casein (Smaco)			18.0	18.0	18.0	18.0			18.0	18.0	18.0	18.0
Casein hydrolysate	18.0	18.0					18.0	18.0				
Brewer's yeast											7.0	7.0
Salts 2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.0	4.0
Vitamin mixture 1	1.0			1.0	1.0	1.0	1.0	1.0		1.0		
Vitamin mixture 2		1.0	1.0						1.0			
Corn oil (Mazola)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cod liver oil									2.0	2.0	2.0	2.0
Percomorph liver oil	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]	2.4[1]				
Choline chloride	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Sulfasuxidine	0.08	0.08	0.08	0.08	1.0		1.0		1.0	1.0		1.0

[1] Drops.

Notes on constituents of diets.

Sucrose: Commercial. Extracted 3 times with about 4 parts by weight of 95% alcohol at room temperature. Twelve hours per extraction.

Casein hydrolysate: A tryptic digest of casein, Amigen, kindly furnished by Mead, Johnson and Co., through the courtesy of Dr. W. M. Cox, Jr. Extracted 15 hours in continuous extractor using ether.

Brewer's yeast: Extracted with ether, similar to procedure for casein hydrolysate.

Sulfasuxidine: Generously supplied by Dr. W. A. Feirer, Sharp and Dohme, Inc.

Vitamins: Most of the synthetic vitamins were kindly furnished through the courtesy of Dr. D. F. Robertson, Merck and Co.

of the ileum and colon are ligated and excised. A purse-string suture is used to close the end of the ileum and of the colon. In order to establish continuity of the alimentary canal a side-to-side anastomosis is made between the terminal ileum and the proximal colon. Connell stitch is used for uniting the openings.

RESULTS

Effect of amino acid source on development of hypoprothrombinemia

When this study was started some uncertainty existed concerning the construction of satisfactory experimental diets deficient in both vitamin K and p-aminobenzoic acid. It was believed that p-aminobenzoic acid might significantly affect the bacteriostatic action of sulfasuxidine, especially if the latter was fed in small amounts (Wood, '42). Since it seemed likely that any p-aminobenzoic acid or vitamin K present in casein could be more thoroughly extracted from the hydrolyzed product, some of the earlier studies (table 2) were made using

TABLE 2

Effect of sulfasuxidine and p-aminobenzoic acid on the prothrombin time and growth of rats fed purified diets.

DIET	NO. OF RATS	AVG. WKS. ON DIET	GROWTH		AVG. PROTHROMBIN TIME			NO. OF RATS WITH 1 OR MORE PTS [1] OVER 50 SEC.
			Max. wt. (avg.)	Gain (avg.)	Min.	Max.	Mean	
			gm.	*gm.*	*sec.*	*sec.*	*sec.*	
1 (Pab [2] —; S [3] 0.08%)	6	10	236	190	37	85	51	1 [4]
2 (Pab +; S 0.08%—)	4	13	262	213	20	85	41	1
3 (Pab +; S 0.08%)	2	9	206	106	32	35	34	0
4 (Pab —; S 0.08%)	3	9	190	84	35	36	36	0
5 (Pab —. S 1.0%)	4	9	82	41	33	105	56	2
6 (Pab —; S —)	2	9	127	89	40	42	41	0
7 (Pab —; S 1.0%)	5	4	116	47	30	70	49	4
8 (Pab —; S —)	5	4	167	101	33	62	42	1
9 (Pab +; S 1.0%)	13	5	117	65	39	51	45	4
10 (Pab —; S 1.0%)	27	5	116	60	54	83	69	16

[1] Prothrombin times.

[2] Para-aminobenzoic acid.

[3] Sulfasuxidine.

[4] Two of the six died of hemorrhages. No prothrombin time determinations were made on these two animals.

diets containing a tryptic digest of casein [3] extracted with ether.

The principal results are in table 2 (compare results from rats fed diets 1 and 2 with those on diets 4 and 3 respectively). Rats fed diets in which ether-extracted casein hydrolysate was the sole source of amino acids had higher prothrombin time values and a higher incidence of hypoprothrombinemia than those fed "vitamin free" casein.[4] No data are available with which strict comparison might be made concerning the effect of the hydrolyzed casein in diet 7. However four of the five rats fed that diet had one or more prothrombin time values above 50 sec. Even one animal fed diet 8, similar to diet 7 except that it contained no sulfasuxidine, developed a mild degree of hypoprothrombinemia. There seems to be no basis for deciding whether the difference between the two sources of amino acids might be attributed to small amounts of vitamin K or p-aminobenzoic acid in the unhydrolyzed casein. However we are convinced from the evidence on hand that "vitamin free" casein can be used satisfactorily in experimental diets to produce vitamin K deficiency in the presence of bacteriostatic agents.

Effect of different levels of sulfasuxidine on prothrombin time

Only two different levels of the sulfonamide were tested, 0.08% and 1.0%. The latter, as might be expected, caused a higher percentage of the animals to develop a prolonged prothrombin time, as shown in table 2. However, certain diets containing only 0.08% of the sulfonamide produced severe hypoprothrombinemia in some of the animals.

Bacterial counts [5]

Bacteriological studies of feces taken from the lower colon and also of the material from the cecum, both obtained at

[3] Amigen.

[4] Smaco.

[5] Grateful acknowledgment is made to Mr. William Roessler, Department of Botany and Bacteriology, for the bacteriological studies.

autopsy, showed a marked reduction in the E. coli content (desoxycholate agar plates) of rats on diets 3, 4, 5, and 7 as compared with the counts from control rats on diets 6 and 8. The bacterial counts were made on rats that had been on the experimental diets for 4 to 9 weeks.

Effect of p-aminobenzoic acid on the action sulfasuxidine

Black, Overman, Elvehjem and Link ('42) found that p-aminobenzoic acid, when added to a purified diet, "counteracted growth inhibition and prolonged prothrombin time" resulting from the presence of sulfaguanidine at a 0.5% level. In a note added concerning sulfasuxidine it is stated that this sulfonamide caused changes in prothrombin time similar to those observed with sulfaguanidine and that the effects were antagonized in a similar manner by vitamin K and liver extract.

Our studies show that the prothrombin time is definitely lower when p-aminobenzoic acid is added to diets containing either 0.08% sulfasuxidine or 1.0%. Also, the incidence of definite hypoprothrombinemia is less in those receiving p-aminobenzoic acid (compare results from animals on diet 1 with those on diet 2, and diet 10 with those on diet 9, table 2). This is a striking difference.

The effect on the growth (table 2) and appearance of the animals is also clearly recognizable. Animals fed p-aminobenzoic acid gained more weight during the experimental period and were less decrepit than those deprived of it. The effect on growth was most marked after the animals had been on the diet for 3 to 4 weeks. Nearly all rats receiving 1% sulfasuxidine without p-aminobenzoic acid lost weight after 3 or 4 weeks, but those receiving an equal amount of the sulfonamide with p-aminobenzoic acid added tended to gain weight, although very slowly. For instance, only one rat in a group of thirteen fed diet 9 (p-aminobenzoic acid added) lost weight after the third or fourth week but twenty-one out of a group of twenty-seven fed diet 10 (p-aminobenzoic acid deficient) lost weight. Also, in the former group the fur was cleaner

and smoother, and the animals were less emaciated. Even the feces of those fed p-aminobenzoic acid were not as soft as those from rats not given the compound.

It seems probable that the bacteriostatic effectiveness of a sulfonamide is due to its competition with p-aminobenzoic acid for an important enzyme site on the bacterial cell (Wood, '42). In view of this it might be supposed that animals fed only 0.08% sulfasuxidine would show less growth inhibition than those fed 1.0% since the content of p-aminobenzoic acid was the same in all diets to which it was added. Our data are not sufficient to clarify this point but they do indicate that growth inhibition is greater in those fed the higher level of the sulfonamide (table 2).

Effects of cecectomy

Four main groups of animals on the basal vitamin K-free diet were studied: group (a), cecectomized and fed 1.0% sulfasuxidine (diet 12); group (b), cecectomized and fed no sulfasuxidine (diet 11); group (c), unoperated and fed 1.0% sulfasuxidine (diet 12); group (d) unoperated and fed 1.0% sulfasuxidine plus 75 mg. 2-methyl-1, 4-naphthoquinone per 100 gm. of diet (diet 12K).

Table 3 shows the principal results. A high incidence of hypoprothrombinemia occurred when sulfasuxidine was fed to cecectomized rats while the incidence of hypoprothrombinemia was low when the sulfonamide was not included in the diet of similarly operated animals. The prothrombin time was elevated in fourteen out of the seventeen rats in group (a). Out of nine in group (b) only two had prothrombin times that were higher than normal, and these were only slightly elevated. The average prothrombin time of cecectomized rats fed sulfasuxidine was 68 sec. The average for those cecectomized but not fed sulfasuxidine was only 38 sec., which, according to our procedure, is essentially normal. Moreover, nearly all the rats in the former group eventually died of pericardial hemorrhages following cardiac puncture, but none of the latter

group succumbed or showed any signs of hemorrhage following cardiac puncture.

In addition, animals fed sulfasuxidine and the same basal diet (diet 12), but with intact ceca, group (c) showed scarcely any evidence of vitamin K deficiency (table 3). One of the seven in this group had a slightly elevated prothrombin time during the latter part of the experiment but this soon returned to normal. Another rat in this group, not represented in the table, suddenly became prostrate and was found to be suffering from an internal hemorrhage. The prothrombin time of

TABLE 3

Effect of cecectomy on the prothrombin time of sulfasuxidine-fed rats on a vitamin K-free diet.

DIET NO.	NO. OF RATS	AVG. WEEKS ON DIET	AVG. PROTHROMBIN TIME (PT)				TOTAL NO. OF PTS [1] DETNS	NO. OF PTS [1] OVER 50 SEC.	NO. OF RATS WITH 1 OR MORE PT OVER 50 SEC.
			4–6 weeks sec.	7–9 weeks sec.	10–12 weeks sec.	Mean sec.			
Cecectomized									
12	17	10.0	63	68	74	68	72	35	14
11	9	10.3	35	36	43	38	29	2	2
Not cecectomized.									
12	7	11.5		36	39	38	14	1	1
12K [2]	11	10.5	33	41	35	36	27	1 [3]	1 [3]

[1] Prothrombin time (or times).

[2] 75 mg. 2-methyl-1, 4-naphthoquinone added per 100 gm. diet.

[3] The actual prothrombin time was 51 sec.

this animal was not determined. Its blood did not clot and the hemorrhage was attributed to prothrombin deficiency. Therefore, two animals out of a group of eight had prothrombin deficiency, an outcome almost identical with that of cecectomized rats not fed sulfasuxidine.

A total of eleven control rats with ceca intact received diet 12K (group (d), table 3). Twenty-seven prothrombin time determinations were made. The average was 36 sec. Control animals, not fed the synthetic vitamin K, had an average of 38 sec. This is additional evidence that, with the type of diet

used, intact (unoperated) animals fed sulfasuxidine tend to maintain essentially normal prothrombin levels. However, one animal fed the synthetic vitamin K had one prothrombin time slightly prolonged, namely 51 sec. The two succeeding prothrombin times of this rat were in the normal range. Whether the unexpected high value was actually due to decreased prothrombin content at the time or was caused by some technical error cannot be decided.

The growth and appearance of the cecectomized animals was normal, being similar to that of unoperated controls. Apparently the diets (11 and 12) were adequate except for vitamin K.

TABLE 4

Effect of 2-methyl-1, 4-naphthoquinone (menadione) on the prothrombin time of individual rats.

DIET NO.	TREATMENT	SEX	WKS. ON DIET BEFORE FEEDING MENADIONE	PROTHROMBIN TIME		DAYS AFTER FEEDING MENADIONE
				Before feeding menadione	After feeding menadione	
				sec.	*sec.*	
12	unoperated	♂	7	101	45	7
12	unoperated	♀	7	62	43	7
12	cecectomized	♂	7	60	38	7
12	cecectomized	♂	7	89	38	14
12	cecectomized	♂	12	73	31	2
12	cecectomized	♂	12	45	34	3
12	cecectomized	♂	11	77	32	2
11	cecectomized	♂	10	40	36	2
11	cecectomized	♂	10	37	30	3
9	unoperated	♀	6	70	30	7

Alleviation of hypoprothrombinemia with 2-methyl-1, 4-naphthoquinone

The hypoprothrombinemia, produced under the above described conditions, was undoubtedly due to vitamin K deficiency. In table 4 this is shown by data from ten different rats. After the experiment had continued 6 to 12 weeks synthetic vitamin K was added at a level of 75 mg. per 100 gm. of diet. In animals with elevated prothrombin time normal values were obtained within 2 to 7 days after administration of synthetic vitamin K (table 4).

DISCUSSION

It is significant that so much variability should occur in the incidence of vitamin deficiency when groups of rats are fed sulfasuxidine. This has been noted in all our studies dealing with this sulfonamide. For instance, three of seventeen cecectomized rats given sulfasuxidine, but no vitamin K, remained totally without evidence of hypoprothrombinemia for the duration of the experiment, 10 to 12 weeks, even though the prothrombin time of all the remaining fourteen became greatly elevated. Such variability in response does not occur in animals fed diets deficient in factors which cannot be synthesized in the intestinal tract, i.e., inorganic elements. It indicates that there is a variable degree of vitamin synthesis by intestinal microorganisms as well as variation in coprophagy and other factors promoting the acquirement of certain vitamins. Light et al. ('42) found marked variability in the growth response of rats fed a vitamin B complex-free diet containing 0.5% sulfaguandidine. A somewhat similar effect was reported by Woolley ('42) who observed occasional spontaneous alleviation of inositol deficiency in mice. The intestinal flora of such animals were capable of synthesizing inositol in vitro whereas in those mice continuing to exhibit symptoms of inositol deficiency the flora could not effect such synthesis.

The difference between ether-extracted casein hydrolysate and "vitamin-free" casein in the production of hypoprothrombinemia cannot be satisfactorily explained. It seems probable that the difference might be due to differences in the effect of the two sources of amino acids on the activity of the intestinal microorganisms which synthesize vitamin K. In some unreported experiments rats fed unextracted casein hydrolysate as a source of amino acids seemed to show as high an incidence of hypoprothrombinemia as those given the extracted material. This seems comparable to the findings that raw starch, dextrin or lactose are more conducive to the synthesis of certain B complex vitamins than glucose or sucrose. (Guerrant et al., '35; Tange, '39; Taylor et al., '42).

The data reported here should be considered in connection with the recent studies of Gant et al. ('43) on the concentration of E. coli in the feces and ceca of rats fed purified diets containing sulfasuxidine for different periods of time. In their studies the E. coli count decreased within 3 days after giving sulfasuxidine and remained low for 10 days. Thereafter it began to increase. After 5 weeks it was near the normal level in rats not given a source of "folic acid", but in those which did receive "folic acid" the count remained low. Possibly in our animals given purified diets, which were low in "folic acid", synthesis of vitamin K after the fourth or fifth week was sufficient to account for the failure of hypoprothrombinemia to occur in some. However, Welch and Wright ('43) report that the prothrombin time is decreased by the addition of "folic acid" to the diet of rats fed sulfasuxidine. It should be noted that most of our cecectomized rats receiving sulfasuxidine had high prothrombin time as late as the twelfth week and several eventually died of hemorrhages. These animals were undoubtedly receiving considerable amounts of "folic acid" because the basal diet contained 7% yeast. If some form of resistance to sulfasuxidine tends to develop in the intact rat fed the drug and is manifested by an approach to normal in the activity of bacteria such as E. coli, our data indicate that such does not occur within a period of 12 weeks in cecectomized rats. Hence any tendency toward sulfonamide resistance, possibly through increased synthesis of p-aminobenzoic acid (Landy et al., '43), might be greatly reduced in degree by removal of the cecum or other loci of great bacterial activity.

We did not study the well established inhibiting effect of p-aminobenzoic acid on the bacteriostatic action of sulfaguanidine (Black, McKibbin and Elvehjem, '41), but we did find that the action of sulfasuxidine is antagonized by p-aminobenzoic acid. However, the practically complete absence of hypoprothrombinemia in rats fed ether-extracted yeast and sulfasuxidine cannot be satisfactorily explained on the basis of the p-aminobenzoic acid present in such yeast since it prob-

ably furnished far less p-aminobenzoic acid than was fed in the purified diets (Blanchard, '41). If the yeast exerted its effect by promoting vitamin K synthesis in the alimentary tract the action might have centered in the cecum because cecectomized animals fed the yeast-containing diet and sulfasuxidine had a high incidence of hypoprothrombinemia, whereas the incidence in unoperated control rats was practically nil.

Our results confirm the findings of Griffith ('35) and of Taylor et al. ('42) that rats receiving a good diet do not appear to be handicapped by absence of the cecum. Some of our animals on diet 11 were observed under experimental conditions for about 12 weeks without discernible abnormality. Moreover, certain of these were maintained on a stock diet for over 12 additional weeks without any deviation from normal. Marked hypertrophy and dilatation of the ileum and colon at the site of the anastomosis occurred in the cecectomized animals. This confirms the observation of Griffith ('35) and of Taylor et al. ('42).

That the cecum, or comparable structures favorable to bacterial activity, may be indispensable under some circumstances is clearly shown. The results indicate that, unless the diet is adequate, severe deficiency states may occur when bacterial activity is reduced in the intestinal tract through the bacteriostatic effect of sulfonamides and/or the absence of natural "fermentation vats" such as ceca or rumina.

SUMMARY

Young cecectomized rats fed 1% sulfasuxidine in a vitamin K-free diet containing yeast show a high incidence of severe hypoprothrombinemia. Similarly operated animals fed the basal diet alone, without sulfasuxidine, exhibit a very low incidence of hypoprothrombinemia. Likewise, unoperated rats fed the same basal diet with sulfasuxidine added show only a low incidence of hypothrombinemia. The hypoprothrombinemia is readily alleviated by feeding 2-methyl-1, 4-naphthoquinone. Cecectomized rats appear to maintain good nutritional status if the diet is fully adequate. The data show that

the cecum is an important site of vitamin K synthesis but that this vitamin can be formed in other parts of the intestinal tract.

Young rats fed 1% sulfasuxidine in a purified diet containing B vitamins but no p-aminobenzoic acid develop hypoprothrombinemia and show great restriction in growth. The addition of p-aminobenzoic acid markedly reduces the incidence of hypoprothrombinemia and decreases the restriction on growth. This indicates that p-aminobenzoic acid partially counteracts the effect of sulfasuxidine on vitamin synthesis in the intestinal tract.

Hypoprothrombinemia and impairment of growth may occur to some extent in young rats fed vitamin K-free purified diets containing only 0.08% sulfasuxidine. These effects are more pronounced when the level of sulfasuxidine is raised to 1%.

Hypoprothrombinemia is more prevalent when hydrolyzed casein, instead of "vitamin-free" casein, is the source of amino acids in purified diets containing sulfasuxidine.

ACKNOWLEDGMENT

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FURTHER STUDIES ON THE COMPARATIVE VALUE OF BUTTER FAT, VEGETABLE OILS, AND OLEOMARGARINES [1]

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Data showing the superiority of butter fat as compared with the vegetable oils for the growth of young rats have been published previously (Schantz, Elvehjem and Hart, '40). This experimental work was carried out with raw skimmed milk as the basal portion of the ration into which were homogenized the fats at a 4% level. Experiments have also been completed with an ether-extracted skim milk powder as the basal portion of the ration (Boutwell, et al., '43). This extraction procedure was followed in order to minimize the amount of butter fat in the vegetable oil rations. Distinct differences in growth favorable to butter fat were obtained. Studies on the effect of various carbohydrates demonstrated that when the lactose was replaced by other carbohydrates, such as sucrose, dextrose, starch, or dextrin, the superiority of butter fat as compared to corn oil disappeared. The ration employed for these comparisons contained 32% of carbohydrate and 28% of fat.

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Unpublished work from this laboratory showed that as the level of lactose was increased, the difference in growth rates between rats fed butter fat and those fed corn oil became greater, and therefore, a ration having 48% carbohydrate was used in the present studies. This level of lactose is between the 32–40% found in cow's milk and the 50–55% found in human milk (dry basis). Various vegetable oils and animal fats including butter fat were studied on this ration when the carbohydrate portion was entirely lactose, and when a mixture of carbohydrates was substituted. This mixture was modeled after the mixed carbohydrate diet such as man ordinarily consumes, and the results of this work led to an investigation of oleomargarines on similar rations.

EXPERIMENTAL

The ration used consisted of:

Carbohydrate	48%
Fat	28%
Casein (fat free)	20%
Salts IV[2]	4%

Vitamins added per 100 gm. of ration:

	mg.
Thiamine	0.500
Riboflavin	0.500
Nicotinic acid	0.625
Pyridoxine	0.625
Calcium pantothenate	5.000
p-Aminobenzoic acid	30.00
Inositol	100.0
Choline chloride	250.0
Calciferol	.014
β-carotene	.560
α-tocopherol	2.24
2-methyl-1, 4-naphthoquinone	.21

Extraction of the casein with boiling 95% ethanol for two 4-hour periods, followed by two exhaustive diethyl ether extractions, insured against foreign fat contamination. The rations were made up weekly and stored under refrigeration

[2] Phillips, P. H., and E. B. Hart. J. Biol. Chem. vol. 109, p. 657, 1935.

between daily feeding periods. Ad libitum feeding was practiced.

The experiments with the various vegetable oils and animal fats were set up in two parallel series, each consisting of nine groups of six male 21-day-old rats of the Sprague-Dawley strain. One of the series received lactose as the sole carbohydrate, and the other received as the 48% carbohydrate portion a mixture with the following constitution: lactose 3, sucrose 15, dextrose 5, starch 15, and dextrin 10 parts respectively. One of the following fats or oils was fed to each of the nine groups

TABLE 1

Average weights at the end of 5 weeks on both the lactose and mixed carbohydrate rations. The figures represent the average weight in grams of the six male rats in each group.

	BUTTER FAT	CORN OIL	COCONUT OIL	COTTONSEED OIL	SOYBEAN OIL	OLIVE OIL	PEANUT OIL	CRISCO	LARD
Lactose ration	169	136	154	155	138	139	138	156	163
Mixed carbohydrate ration	199	193	219	200	204	184	199	200	202

of animals in each series: butter fat obtained by decantation from fresh unsalted sweet-cream butter from the University of Wisconsin Creamery, corn oil[3], coconut oil, cottonseed oil (winter-pressed), soybean oil, peanut oil,[4] olive oil, hydrogenated cottonseed oil[5]. Table 1 shows the results of this experiment expressed as the average weight of the six rats in each group at the end of 5 weeks. While the records shown are for six male rats in each group, we have data for twenty-four animals on both butter fat and corn oil-lactose rations. The average results are not different from the figures on six male rats given in this paper.

[3] Mazola.

[4] Planter's Hi-Hat.

[5] Crisco.

In order to determine whether or not the difference in weight between the butter fat and corn oil groups on the lactose regime was due to the deposition of more fat in the case of the butter fat group, the six rats from each group were killed, the gastro-intestinal tracts removed, and after pooling the carcasses into two respective groups, analyzed for total fat, protein and water content. A similar procedure was followed with the rats fed the butter fat mixed carbohydrate ration to determine whether the kind of carbohydrate fed influenced the relative amounts of these constituents, and therefore afforded a partial explanation for the superior growth on the mixed carbohydrate rations. These data are given in table 2.

TABLE 2

Results of gross analysis of rats. Each figure represents the per cent obtained by pooling the six rats in each group.

RATION	MOISTURE	FAT (DRY BASIS)	PROTEIN (DRY BASIS)
	%	%	%
Butter fat mixed carbohydrate ration	61.6	39.2	51.6
Butter fat lactose ration	65.1	34.8	53.5
Corn oil lactose ration	65.2	32.8	55.0

Using the experimental procedure described above, various commercial oleomargarines were compared to butter fat and corn oil on both the lactose and mixed carbohydrate rations. The oleomargarines were freed of water and non-fatty material by decantation, similar to the preparation of the butter fat. Six male rats 21 days of age were placed on each of the following fats and oils: butter fat, corn oil, three different oleomargarines (oleos A-1, A-2, and A-3) labeled by the manufacturer to be mixtures of animal fats, and four oleomargarines (oleos V-1, V-2, V-3 and V-4) labeled to be mixtures of hydrogenated vegetable oils. One such series of nine groups of animals received lactose as the sole carbohydrate, while in a parallel series of nine groups the carbohydrate mixture already described was fed. Ad libitum feeding was

practiced, and consumption records were kept. Table 3 gives the results of these experiments in terms of the average weight of the six rats in each group at the end of 6 weeks. Table 4 shows the results of the food consumption records expressed in terms of per cent efficiency. These figures were obtained by

TABLE 3

Average weights in grams at the end of 6 weeks of rats fed butter fat, corn oil, and various oleomargarines on both the lactose and mixed carbohydrate rations. Each figure represents the average of six male rats.

	BUTTER FAT	CORN OIL	OLEO A-1	OLEO A-2	OLEO A-3	OLEO V-1	OLEO V-2	OLEO V-3	OLEO V-4
Lactose ration	219	177	212	214	206	212	202	193	194
Mixed carbohydrate ration	241	224	252	240	224	242	224	246	250

TABLE 4

Per cent efficiency for rats fed butter fat, corn oil, and various oleomargarines on lactose and mixed carbohydrate rations.

$$\text{Per cent efficiency} = \frac{\text{grams gain}}{\text{grams consumed}} \times 100$$

PERIOD	CARBOHYDRATE	BUTTER FAT	CORN OIL	OLEO A-1	OLEO A-2	OLEO A-3	OLEO V-1	OLEO V-2	OLEO V-3	OLEO V-4
1 to 3 weeks inclusive	Lactose	42	29	43	33	37	35	35	32	33
	Mixture	45	44	46	45	43	44	46	44	47
1 to 6 weeks inclusive	Lactose	39	36	41	34	37	37	35	32	34
	Mixture	41	41	42	40	39	40	39	42	41

dividing the number of grams gained during a given period by the number of grams of food consumed during that period and multiplying by 100. The periods chosen for this report are the first to third weeks inclusive and the first to sixth weeks inclusive.

DISCUSSION

The data given in table 1 clearly show that from a nutritional standpoint, an interrelationship between the kind of carbohydrate and the kind of fat exists, and this fact must be considered in obtaining the correct nutritional value of either

nutrient. With lactose as the sole carbohydrate, the vegetable oils were definitely inferior to butter fat and lard. Thus the rats receiving butter fat on the lactose diet grew 33 gm. more during a period of 5 weeks than those fed corn oil. The other vegetable oils tested ranged from 15 to 32 gm. below butter fat, while lard, which is an animal fat, gave a figure of only 6 gm. below butter fat. These results substantiate our earlier observations (Boutwell, et al., '43) that with lactose as the only carbohydrate, animal fats including butter fat were superior to vegetable oils in the nutrition of young rats. That the kind of carbohydrate is important can also be seen from table 1. With the mixture of carbohydrates substituted for lactose, approximately equal growth was obtained by the rats fed either animal fats or vegetable oils, and furthermore, growth for each group was much greater on the mixture than on lactose. No apparent reason can be given for the superior growth of the rats fed coconut oil nor the inferior growth of the rats fed olive oil when the carbohydrate mixture was used. Table 3 shows that the only marked difference in gross analysis between the rats fed lactose and those fed the mixture of carbohydrates was in the total per cent of fat. On the carbohydrate mixture the butter fat animals averaged 39.2% of fat, while on the lactose regime the butter fat and corn oil groups averaged 34.8 and 32.8% respectively. This can be interpreted as meaning that on the carbohydrate mixture more carbohydrate is available for fat formation than on the lactose diets, and that perhaps more fat is formed and deposited than when lactose is the sole carbohydrate in the ration. Such an interpretation could account in part for the superior growth on the carbohydrate mixture in comparison to that on lactose. The differences in gross analysis between the butter fat and corn oil groups on the lactose ration are not sufficiently large to account for the superior growth of butter fat fed rats in comparison to those fed corn oil, and by analogy, in comparison to the animals fed any of the vegetable oils tested.

These data are related directly to the question of the desirability of allowing the manufacture of a "filled milk" which may become the sole source of nutrition for the growing child. The data make it apparent that a great responsibility would be thrown on those who believe that such a product as "filled milk" as the sole article of nutrition could function adequately in early nutrition.

Unpublished data have shown definitely that "filled milks" are inferior to whole milk in growth promoting properties when fed to young rats. By a "filled milk" we mean a skimmed milk fortified with a vegetable oil to the same fat level as a whole milk.

It is evident from the above data that the results obtained on the mixed carbohydrate diet would conform more closely to the carbohydrate intake of the ordinary human diet, than would the results on the lactose diet. In the mixed diet of man starches, sugars and a variety of other carbohydrates are consumed. Potatoes, breads, cane sugar, and molasses all contribute starch, sucrose, or dextrose to the diet. Consequently, it becomes possible to throw some light on the much discussed question as to the comparative nutritive value of oleomargarines of either vegetable or animal origin when reinforced with vitamin A and fed in a ration complete in all other nutrients.

Table 4 shows that with lactose as the sole carbohydrate the animal oleomargarines were but slightly below butter fat in nutritive value, while all but one of the vegetable oleomargarines were distinctly inferior. The poorest growth was obtained with the corn oil ration. These results are in keeping with the thesis that the animal fats investigated are superior to the vegetable oils for the early growth of rats on a lactose ration. On the mixed carbohydrate diet, such as man ordinarily consumes, there was no conclusive evidence that oleomargarines were inferior to butter fat. In fact, some oleomargarines showed slight superiority and there was quite as much variation among oleomargarines as between the butter fat and the oleomargarines. If 6 weeks of growth

of the rat at a rapid stage of its development can be used as the criterion of adequate nutrition, then certainly these oleomargarines compare favorably with butter fat. Whether this deduction is entirely sound can be answered only when growth to maturity and reproduction are studied experimentally.

The results given in table 4 show that on the lactose diet the rats receiving butter fat, oleo A-1 or oleo A-3 were more efficient in their utilization of food during the first to third week period in comparison to those receiving corn oil or one of the other oleomargarines tested. Thus all but one of the fats of animal origin were superior on an efficiency basis to those of vegetable origin during this early period. At the end of 6 weeks the picture remained relatively the same, though the corn oil and oleo V-1 groups had improved somewhat. With the carbohydrate mixture in the diet, however, there were no marked differences in efficiencies between the various groups and in all cases the efficiency values were higher than the corresponding group receiving only lactose as the carbohydrate. The over-all picture in respect to efficiency data is, therefore, similar to that shown by the growth records. The higher efficiency of the rats on the mixed carbohydrate diet plus the fact that more fat was deposited than in the case of the lactose rations (table 2) strongly indicate that the mixed carbohydrate is more readily utilized, and if such is the case, more fat is synthesized, with the end result that the importance of the dietary fat is lessened and all groups grew the same. A more detailed investigation is necessary before a definite conclusion can be reached on this phase of the problem.

SUMMARY

1. With lactose as the sole carbohydrate in the diet, rats showed superior growth when fed butter fat or lard as compared to corn oil, coconut oil, cottonseed oil, soybean oil, peanut oil, olive oil and hydrogenated cottonseed oil.

2. With a mixture of carbohydrates composed of sucrose, starch, dextrose, dextrin, and lactose in the diet, the average

growth response of the animals fed vegetable oils was equal to that of the animals fed butter fat and lard. The growth rate on this ration was more rapid than when all of the carbohydrate was present as lactose.

3. Properly fortified oleomargarine fats gave growth equal to butter fat over a period of 6 weeks when the above mixture of carbohydrates was incorporated in the rations.

4. Properly fortified oleomargarines did not give growth equal to butter fat when lactose was the sole carbohydrate in the diet. On such a regime rats fed butter fat grew slightly better than rats fed oleomargarines of animal origin, but decidedly better than rats fed oleomargarines of vegetable origin.

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THE EFFECT OF HIGH TEMPERATURE AND B-VITAMIN LEVELS OF THE DIET UPON THE METABOLISM AND BODY COMPOSITION OF RATS[1]

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Mills ('41 and '42) has reported that for optimal growth of rats maintained at 91°F. and fed ad libitum, the concentrations of thiamine, choline and pyridoxine needed in the diet were greater than those required at 65°F. The optimal levels of riboflavin and pantothenic acid were the same at both temperatures.

In the present work, the effect of a high environmental temperature upon the metabolism and utilization of some of the B vitamins in the rat were studied in an attempt to gain more information concerning the requirements for and utilization of these B vitamins at high temperatures. Growth, urinary excretion of nitrogen and some B vitamins and body composition were determined in four groups of rats maintained at 75° and 91°F. on diets containing two levels of B vitamins, one slightly above minimal requirements, the other greatly in excess. The food intake of the groups at lower temperature was restricted to that of the corresponding groups at 91°F., which were fed ad libitum.

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[2] The authors wish to thank Isabella King for her helpful technical assistance.

EXPERIMENTAL

Twenty male rats (age 30 days) weighing 60-91 gm. were selected for four groups of five, each group similar in distribution of weights and averaging 71 gm. per rat. The groups were placed in metabolism cages and fed purified diets low and high in the vitamin B-complex. The diets were made up as follows: purified casein[3], 180 gm.; corn starch, 220 gm.; sucrose, 420 gm.; beef suet, 80 gm.; cod liver oil, 40 gm.; B. D. H. mixture[4], 40 gm.; ruffex[5], 20 gm.; choline hydrochloride, 2.5 gm.; and concentrated liver extract[6] in such amounts as to provide for the "low vitamin" and "high vitamin" diets, respectively, 2 and 4 mg. of thiamine, 2 and 4 mg. of riboflavin, 4 and 8 mg. of pantothenic acid, 6.6 and 13.2 mg. of nicotinic acid, and 1.4 and 2.8 mg. of pyridoxine. Water was added to give 5 kg. of cooked diet. The diets were cooked as previously described (Sarett, Klein and Perlzweig, '42; Sarett and Perlzweig, '43) and contained 2 levels of the vitamin B-complex supplied as a supplemented liver concentrate. The choline content was the same for both diets. Groups A and B received the diet high in B-complex and groups C and D the lower vitamin diet.

All four groups were kept at room temperature (75°F.) for 5 days during which body weights and urine analyses were checked to determine the extent of agreement of the results yielded by similar groups. At this time the average weight of the rats in groups A, B, C and D were 104, 106, 99 and 101 gm. respectively. The cages containing groups A and C were then transferred to a large insulated dog cage which was maintained at 90–91°F. by means of covered electric bulbs, two of which were attached to a thermostatic control. No attempt was made to regulate or increase the relative humidity which

[3] Labco.

[4] Prepared according to Drummond and Watson ('22).

[5] Fisher Scientific Company.

[6] Generously supplied by Lederle Laboratories, Pearl River, New York. The extract of each 10 gm. of fresh liver was supplemented with synthetic vitamins to contain the following: thiamine, 1 mg.; riboflavin, 1 mg.; calcium pantothenate, 2 mg.; nicotinic acid, 3.3 mg.; and pyridoxine, 0.7 mg.

was usually about 20%. Fresh water and 2% NaCl solution were available to all four groups of animals. Saline intake is reported in table 1.

The food intakes of the animals at room temperature (B and D) were restricted to the previous day's consumption by the corresponding group at high temperature (A and C). The animals at room temperature were therefore eating less than they would have eaten normally. The high vitamin group at high temperature (A) ate about 25% more food than the low vitamin group at high temperature (C). Since the B

TABLE 1

Food intake and weight gain per rat per day during experimental period at 75° and 91°F.

GROUP	WEIGHT GAIN	INTAKE							
		Calories	Protein	Thiamine	Riboflavin	Pantothenic acid	Nicotinic acid	Choline	2% NaCl
	gm.	*cal.*	*gm.*	*μg.*	*μg.*	*μg.*	*μg.*	*mg.*	*ml.*
A–High Vitamin, 91°F.	3.6	37.4	1.53	37.7	37.7	75.4	124	21	13
B–High Vitamin, 75°F.	3.1	37.5	1.53	37.9	37.9	75.8	125	21	13
C–Low Vitamin, 91°F.	2.2	28.4	1.15	14.5	14.5	29.0	48	16	18
D–Low Vitamin, 75°F.	1.6	28.6	1.17	14.6	14.6	29.2	48	16	24

vitamin concentration of the high diet was twice that of the low diet, the actual vitamin intake of groups A and B was about two and one-half times the vitamin intake of groups C and D. The average daily food intake and weight gain during the experimental period are given in table 1. On the twenty-second day, groups A and B were fasted for 24 hours and then sacrificed for analysis. Groups C and D were analyzed similarly after the twenty-fifth day.

Urines were collected daily and analyzed for nitrogen, riboflavin, panothenic acid, nicotinic acid and trigonelline. Nitrogen was determined by Kjeldahl method, nicotinic acid and trigonelline by the method of Perlzweig, Levy and Sarett ('40) and pantothenic acid and riboflavin by microbiological methods (Pennington, Snell and Williams, '40, Snell and Strong, '39). In the pantothenic acid assay the yeast supple-

ment of Strong, Feeney and Earle ('41) was substituted for the alkali treated yeast extract.

Sacrifice of the animals and preparation of liver and carcass for analysis were carried out as previously described (Sarett and Perlzweig, '43). Nicotinic acid was determined chemically (Dann and Handler, '41), nitrogen by Kjeldahl, and fat by alkaline digestion, acidification, extraction with ether and weighing. Riboflavin and pantothenic acid were determined microbiologically as in the urine, after digestion with papain and takadiastase at pH 4.5 and filtration through supercel (Cheldelin et al., '42). The filtrates were extracted with ether to remove interfering fats (Bauernfeind, Sotier and Boruff, '42) before the pH was adjusted for the assays. For thiamine the thiochrome method of Mason and Williams ('42) was directly applied to a portion of these enzymatic digests without the use of permutit. A slight excess of ferricyanide was used to insure complete oxidation to thiochrome and checked by recoveries of added thiamine. Blanks were obtained by sulfite treatment.

Effect of high temperature upono the urinary excretion of nitrogen and B-vitamins

Table 2 shows the average daily excretion per rat of nitrogen, riboflavin, pantothenic acid, nicotinic acid and trigonelline by each of the four groups during the experimental period.

TABLE 2

Urinary excretion per rat per day of nitrogen and B-vitamins during experimental period at 75° and 91°F.

GROUP	NITROGEN	RIBOFLAVIN	PANTOTHENIC ACID	NICOTINIC ACID AND TRIGONELLINE
	mg.	*μg.*	*μg.*	*μg.*
A–High Vitamin, 91°F.	102	12.9	7.1	281
B–High Vitamin, 75°F.	116	12.2	4.8	277
C–Low Vitamin 91°F.	72	2.1	3.1	188
D–Low Vitamin, 75°F.	87	1.7	2.4	181

A comparison with table 1, which shows the daily intake of these substances, gives an estimate of the amounts retained by the rat. The feces were not analyzed. The nitrogen excretion of the rats at room temperature was higher than that of the corresponding group at 91°F. This was to be expected since the caloric requirements were higher at the lower temperature and is corroborated by the slower growth and lower nitrogen and fat content of the rats at room temperature. However, the rats at high temperatures at both levels of vitamin intake excreted about the same amounts of riboflavin and more pantothenic acid than the corresponding group at room temperature. During the first week the rats at high temperature excreted more riboflavin and pantothenic acid than their respective control groups. For the remainder of the experimental period only the pantothenic acid excretion of the groups on the higher vitamin intake differed markedly; small but insignificant differences were obtained for excreted pantothenic acid at low vitamin intake and for riboflavin at both levels of intake. All four groups excreted more nicotinic acid and trigonelline than their nicotinic acid intake. This extra excretion was about the same for all groups regardless of temperature or B-vitamin content of the diet.

The effect of high temperature upon the body composition of the rat

Table 3 gives the average composition of the livers and carcasses of each group of five rats after the experimental period at 75° and 91°F. Groups A and B were sacrificed after 22 days, C and C after 25 days. The rats kept at high temperature gained more weight than their corresponding control group. The livers of the control groups, however, especially on the high vitamin diet, constituted a greater percentage of the total body weight.

The concentrations of water in the carcasses of the animals kept at 91°F. were significantly lower than in those at 75°F.

TABLE 3

Average composition of rat liver and carcass after experimental period at 75° and 91°F.

GROUP	TISSUE ANALYZED	WEIGHT	WATER	NITROGEN PER GRAM	FAT	ASH	RIBOFLAVIN PER GRAM	PANTOTHENIC ACID PER GRAM	THIAMINE PER GRAM	NICOTINIC ACID PER GRAM
		gm.	%	*mg.*	%	%	*µg.*	*µg.*	*µg.*	*µg.*
A										
22nd day High Vitamin, 91°F.	Carcass	161	62.7	25.5	14.7	3.0	3.5	9.7	1.67	42.8
(5 rats, 183 gm. average weight)	Liver	7.8	74.0	28.8	4.2		23.2	75.7	6.0	132
B										
High Vitamin, 75°F.	Carcass	148	65.4	26.5	12.3	3.3	2.6	7.4	1.35	44.5
(5 rats, 174 gm., average weight)	Liver	9.2	73.2	28.1	4.1		22.	68.9	5.1	124
C										
25th day Low Vitamin, 91°F.	Carcass	134	65.9	26.7	12.3	3.4	2.1	5.4	.82	40.0
(5 rats, 155 gm., average weight)	Liver	6.1	73.6	29.5	4.6		20.	61.	3.9	130
D										
Low Vitamin, 75°F.	Carcass	123	68.4	27.0	8.5	3.6	2.5	6.9	.97	42.5
(5 rats, 140 gm., average weight)	Liver	6.1	73.7	31.1	3.9		21.8	59.4	4.3	129

and were compensated by the increased fat content. The lower fat content at room temperature was presumably due to need for more calories. While the animals of the two groups kept at 91° retained more nitrogen, the concentration of nitrogen in the carcasses of these animals was the same or slightly lower than in their respective control groups. The concentrations of water, fat and nitrogen in the livers of these animals showed no significant differences.

The concentrations of riboflavin, pantothenic acid and thiamine in the carcasses were highest in the group at high temperature at the high level of B-vitamin intake and lowest in the other high temperature group whose diet contained one-half of this B-vitamin level. These differences from the groups at room temperature were significant when analyzed by the t test (Snedecor, '38). Both groups at room temperature had the same concentration of riboflavin and pantothenic acid in their tissues, while the thiamine content varied with the intake. This agrees with the previous observations of Sarett and Perlzweig ('43). The concentrations of riboflavin, pantothenic acid and thiamine in the livers of these animals followed the same pattern as in the carcasses, the only significant differences being that the highest levels of pantothenic acid and thiamine were in group A. The nicotinic acid concentrations were essentially the same in the livers and in the carcasses of all four groups.

DISCUSSION

At levels of intake above the minimum requirements for the rats at room temperature the riboflavin, pantothenic acid and thiamine concentrations in the carcasses of rats kept at high temperature varied with the concentrations of these vitamins in the diet. At room temperature, rats on these diets restricted to the same caloric intake as the respective groups at 91°F. showed no variation in the riboflavin and pantothenic acid concentrations in the carcasses and only the tissue thiamine varied with the level of B-vitamins in the diet. Comparing only groups A and C, which were maintained at

91°F., the data show that the increased B-vitamin content of the diet has increased the appetite and growth rate of the rats, which is curtailed by high temperature (Mills, '41 and '42), and resulted in carcass tissues almost twice as rich in riboflavin, pantothenic acid and thiamine. Given the high vitamin diet, the rats at high temperature laid down tissue containing more riboflavin, pantothenic acid and thiamine per gram than normal rats with this excess B-vitamin intake, while with an intake about adequate at room temperature, the tissues laid down at high temperature are very low in these vitamins. These data on tissue composition may lead to the inference that a level of B-vitamin intake considered adequate at moderate environmental temperatures may result in a partial depletion of such vitamins in the tissues at high temperatures.

The data still cannot be interpreted in terms of requirements of these vitamins at high temperatures, since the divergence in tissue content on the two diets is not correlated with the urinary excretion of these vitamins. On both low and high vitamin diets the rats at high temperature excreted as much or more riboflavin and pantothenic acid than did the respective controls at room temperature. Holt ('43) has also found more thiamine in the urine of human subjects during hot weather. The urinary excretion and total body content of riboflavin and pantothenic acid show that the high vitamin group at high temperature has utilized or destroyed less riboflavin and pantothenic acid than its control group, while the low vitamin group at high temperature has utilized or destroyed more of these substances than its control group. At 91°F. the rats required fewer calories than at 75°F. as evidenced by the higher fat content of the tissues and increased nitrogen retention by the animals. The concomitant need for less vitamins for this catabolism and the finding of increased urinary excretion of these vitamins at high temperature would indicate a lower B-vitamin requirement at higher temperatures, but the opposite conclusions might be

drawn from the tissue concentrations of the vitamins in the rats at high temperature on the low vitamin diet.

The similarity in data for riboflavin, pantothenic acid and thiamine in these experiments may indicate that any changes in requirements, that may be demanded by a change in temperature would be similar for all of these vitamins. Mills ('41, '42), interprets his data on the levels of these vitamins needed for optimal growth of rats at high and low temperatures on a synthetic diet fed ad libitum as indicating no differences in riboflavin and pantothenic acid requirement and a doubling of the thiamine requirements at high temperature.

These findings can only be applied to resting animals at higher temperatures and reveal nothing of the vitamin requirements for work or exercise at high temperature. This obviously important question also requires further investigation.

SUMMARY

Groups of rats were maintained for 22–25 days at 91°F. on high and low intakes of the B-vitamin-complex. The corresponding control groups were kept at 75°F. and their food intake was restricted to that of the animals at 91°. The analyses of the urine and of the bodies at the end of the experiment showed that: At both levels of vitamin intake the rats at 91° gained more weight, retained more nitrogen, fat and water than the corresponding controls. The concentration of water was lower and that of fat was higher in the animals at 91°. The concentration of riboflavin, pantothenic acid and thiamine was highest in the bodies of the group on the high vitamin intake kept at 91° and was lowest in the low vitamin group kept at 91°. In the two groups at 75°, the level of intake had no effect upon the concentration of riboflavin and pantothenic acid in the tissues, while the thiamine concentration varied with the intake. The nicotinic acid of the tissues remained uninfluenced either by level of intake or temperature.

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THE RETENTION OF VITAMINS IN VEAL AND LAMB DURING COOKING [1]

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Considerable interest has developed concerning the vitamen retention in meat after various cooking processes. The vitamin retention in pork during storage, curing and cooking has been reported recently (McIntire et al., '43), (Schweigert et al., '43) and (Schweigert et al., '43a). In this paper we wish to report the thiamine, riboflavin and nicotinic acid retention in veal and lamb after different methods of cooking, namely, roasting, braising, broiling and stewing.

EXPERIMENTAL

The samples of veal used in these studies were obtained from veal carcasses weighing approximately 90 pounds. The lamb samples were obtained from lambs weighing 50 pounds, dressed. The veal samples were prepared as follows: The loin was removed by a cut perpendicular to the aitch bone. One sirloin steak was discarded and two adjacent ¾-inch steaks were retained for cooking tests. The shank was removed from the leg at the stifle joint and the rump was removed from the round. The remaining portion of the leg was used for testing. The third and fourth rib steaks were removed from the shoulder for testing, and a boned rolled cut was prepared

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from the remainder of the shoulder. In all cases paired cuts were obtained from both sides of the animal for fresh and cooked samples.

The stewing meat was obtained by cubing muscular sections of the flanks and shanks. The cubes were than thoroughly mixed and equal portions were used for fresh and cooked samples. Corresponding sirloin, leg, and stewing samples were taken from the lamb carcasses.

The cooking was carried out under standard procedures without seasoning as described in "Meat and Meat Cookery," ('42). The braising method has been described by McIntire et al. ('43). The veal rib and sirloin steaks were braised for 1 hour. Approximately 30 minutes were required to broil lamb steaks. The steaks were placed on a rack 10 inches from the heating unit and when the internal temperature reached 55°C., the steaks were turned and allowed to reach a final internal temperature of 77°C. Roasting time for veal shoulder roasts ranged from 2 hours and 22 minutes to 4 hours and 17 minutes, and for veal leg roast from 3 hours and 45 minutes to 5 hours. The roasting time for leg of lamb was 2 hours and 56 minutes and 4 hours and 32 minutes for the two roasts. Stewing was carried out in a covered iron kettle. The water was heated to boiling and poured on the meat in proportions of 1:2 by weight. The temperature of the stew was allowed to reach 85°C. and maintained for 2 hours.

In every case the drippings and stewing water were retained for analysis. Preparation and storage of the meat samples and drippings and analyses for moisture and fat were carried out as reported by McIntire et al. ('43).

The thiamine content of the samples was determined by the method of Hennessy ('42) with modifications by McIntire et al. ('43). The nicotinic acid was determined by the method of Snell and Wright ('41), after extraction with 4% alkali. The riboflavin determinations were made by the method of Snell and Strong ('39), with some modifications in the digestion and extraction procedure. A suitable sample of meat was autoclaved for 15 minutes in 50 ml. 0.1 N H_2SO_4. Two-

TABLE 1

Detailed analysis of meats. Three series of veal and two series of lamb were analyzed. Illustrative results of the second veal series and the first lamb series.

	LEG OF VEAL FOR ROASTING	VEAL SIRLOIN STEAKS FOR BRAISING	ROLLED VEAL SHOULDER FOR ROASTING	VEAL RIB STEAKS FOR BRAISING	VEAL FOR STEWING	LEG OF LAMB FOR ROASTING	LAMB SIRLOIN STEAKS FOR BROILING	LAMB FOR STEWING
Before cooking								
Wt. of meat — gm.	2640	465	2029	497	548	1241	376	612
H_2O content — gm.	1957	321	1485	368	408	827	231	386
Fat content — gm.	95	53	120	20.0	14	151	75	103
Res. solids — gm.	589	91	424	108	126	250	70	122
Thiamine content — mg.	4.42	0.84	3.45	0.85	0.78	2.48	0.68	0.83
Riboflavin content — mg.	9.1	1.65	7.42	1.93	2.03	3.72	1.06	1.61
Nicotinic acid content — mg.	222	39	142	37.3	41.2	84.2	21.8	34.9
After cooking								
Wt. of meat — gm.	1853	295	1310	297	348	871	276	399
H_2O content — gm.	1133	141	767	175	216	493	142	210
Fat content — gm.	107	57	127	19.6	17.4	131	64	74
Res. solids — gm.	575	87	415	102	114	246	71	106
Thiamine content of meat — mg.	2.34	0.33	1.76	0.29	0.21	1.38	0.49	0.23
Thiamine content of drippings — mg.	0.24	0.16	0.44	0.14	0.19	0.13	0.69	0.20
Riboflavin content of meat — mg.	7.1	1.24	6.2	1.35	1.3	3.26	0.83	1.16
Riboflavin content of drippings — mg.	0.74	0.38	0.89	0.41	0.6	0.36	0.06	0.53
Nicotinic acid content of meat — mg.	171	23.9	109	22	23	58.4	16.8	18.4
Nicotinic acid content of drippings — mg.	35.6	13.0	26.4	9.5	19.0	15.1	2.2	16.2

TABLE 2

Vitamin retention after cooking.

All values in %.

	FRESH WEIGHT RETAINED			THIAMINE RETAINED IN MEAT			TOTAL THIAMINE RETAINED			RIBOFLAVIN RETAINED IN MEAT			TOTAL RIBOFLAVIN RETAINED			NICOTINIC ACID RETAINED IN MEAT			TOTAL NICOTINIC ACID RETAINED		
Determination	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Roast leg of veal	75	69	70	70	53	52	77	59	58	90	78	81	97	86	90	86	77	73	100	93	87
Braised sirloin of veal	63	61	61	45	40	35	68	59	..	69	75	74	89	98	..	60	61	57	95	94	..
Roast shoulder of veal	71	64	68	65	51	53	76	63	66	91	83	84	99	95	96	93	77	78	99	96	97
Braised rib of veal	66	60	62	49	36	35	66	51	51	77	70	72	94	91	94	69	60	60	95	85	90
Stew of veal	63	63	62	22	26	28	47	51	54	69	64	65	103	95	106	51	56	47	101	102	95
Roast leg of lamb	71	70	..	53	56	..	58	61	..	82	88	..	94	97	..	77	69	..	97	87	..
Broiled sirloin of lamb	65	73	..	68	72	..	77	82	..	80	78	..	90	84	..	83	77	..	100	87	..
Stew of lamb	64	65	..	26	28	..	50	52	..	68	72	..	100	105	..	52	53	..	105	99	..

tenths gram of papain dissolved in 5 ml. of 2.5 molar sodium acetate was then added to the digest and the mixture was incubated over night. The digest was then neutralized, filtered, and extracted by shaking with ether, (Strong et al., '42), then the aqueous portion was made to a definite volume. The papain digest was used because of its low riboflavin content as compared to clarase.

TABLE 3

Table of analysis of fresh and dry veal and lamb samples.

All values μg. per gram.

	THIAMINE		RIBOFLAVIN		NICOTINIC ACID	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
Veal — average of three series of samples.						
Fresh leg	1.8	6.8	3.1	11.7	75	283
Roast leg	1.5	3.9	3.9	10.5	83	222
Fresh sirloin	1.9	6.2	3.1	10.1	71	237
Braised sirloin	1.2	2.4	3.7	7.3	69	138
Fresh shoulder	1.7	6.3	3.2	11.4	61	220
Roast shoulder	1.4	3.5	4.0	9.9	71	174
Fresh rib	1.7	6.5	3.3	12.4	62	233
Braised rib	1.1	2.5	3.9	9.3	65	155
Fresh stew	1.6	6.1	3.1	11.7	68	255
Cooked stew	.66	1.7	3.2	8.5	57	150
Lamb — average of two series of samples.						
Fresh leg	2.0	6.50	2.95	9.6	63	204
Roast leg	1.55	3.60	3.54	8.2	65	151
Fresh sirloin	1.72	4.78	2.80	7.0	53	138
Roast sirloin	1.74	3.52	3.20	6.5	61	123
Fresh stew	1.39	3.48	2.70	7.8	58	166
Cooked stew	.59	1.28	2.93	6.4	47	203

Cooking tests have been carried out on three groups of veal samples and two groups of lamb samples. All analyses were made directly on the fresh cooked and uncooked samples. The vitamin content of the dry samples was calculated on the basis of the moisture analysis. Detailed analyses of veal and lamb samples are shown in table 1. The average per cent retention of weight and vitamins for each method of cooking is shown in table 2. The average vitamin contents of the samples from the three veal series and two lamb series are shown in table 3.

All calculations were made according to the method described by McIntire et al. ('43), and Schweigert et al. ('43).

DISCUSSION

The proximate analyses indicate that almost the entire decrease in weight in the meat during cooking was due to loss of water. Meats roasted and broiled consistently retained more moisture than those braised and stewed. The average range of weight retention for roasting and broiling was from 68 to 71%, while it was 61 to 65% for braising and stewing.

Results for vitamin retention corresponded with water loss in every case. Roasting and broiling procedures resulted in higher retention of the vitamins in the meat than did braising and stewing. The average retention of thiamine after roasting of lamb and veal was 57%; after broiling of lamb, 70%; after stewing of veal and lamb, 26%; and after braising of veal, 40%. The average retention of riboflavin after roasting of veal and lamb was 79 to 86%; after broiling of lamb, 79%; after braising of veal, 73%; and after stewing, 66%. The nicotinic acid retention in the meat was generally lower than riboflavin but higher than thiamine. The average retention of nicotinic acid after roasting was 73% for lamb and 81% for veal; after broiling lamb, 80%; after braising veal, 61%; and after stewing, 52%. These results indicated that nicotinic acid was more loosely bound and more readily extracted than riboflavin.

Average total retention of the vitamins is similar after roasting and braising. The total retention of thiamine after roasting of lamb was 60%, after roasting of veal was 67%; after braising the total retention was 60%. Highest total retention of thiamine resulted after broiling. Eighty per cent of the thiamine was retained after broiling. This supports the results obtained after frying (pan-broiling) of pork by Schweigert et al. ('43). Stewing on the other hand resulted in the lowest total retention of thiamine. Only 51% of the thiamine was retained. This indicates the ability of thiamine thiamine was retained. This indicates the lability of thiamine to heating in aqueous solutions. Total retention of the more

stable vitamins was nearly the same in all cases. The average total retention for riboflavin after the various cooking methods ranged from 87 to 101% and for nicotinic acid from 92 to 100%.

The high total retention of the riboflavin and nicotinic acid indicates that practically all of the loss in the meat alone was due to leaching and extraction. This is borne out by the previously mentioned fact that the vitamin retention closely parallels the moisture retention during cooking. The extent of the vitamin retention can also be correlated with the size of the cuts of meat, the type of cooking and the time required for cooking.

About the same amount of vitamins was retained in the meat after roasting and broiling. The broiled meat was cooked only 30 minutes as compared to 2 to 5 hours required for roasting. However, the steaks are considerably smaller and have much more surface area exposed per unit of weight. The thiamine content of the drippings for the size of the samples was lowest after roasting. This may be attributed to the destruction during the long cooking period. The braising process involves a continual extraction of the meat by the condensing vapors in the kettle. The stewing process is simply a hot water extraction. This likely accounts for the lower vitamin retention in the meats after braising and stewing. Stewing gave the most consistent results of any of the methods studied. This is attributed to more uniform sampling and a more standardized cooking procedure.

The results of the retention studies on veal and lamb are in good agreement with those previously reported on pork (McIntire et al., '43) and (Schweigert et al., '43).

The values obtained for thiamine, nicotinic acid and riboflavin contents of veal and lamb are generally in good agreement with those reported by previous workers. Thiamine values ranged from 1.28 to 2.32 μg. per gram for veal and from 1.35 to 2.0 μg. per gram of fresh lamb. These figures are lower than 3.5 μg. per gram of fresh veal and 3.0 μg. per gram of lamb reported by Waisman and Elvehjem ('41).

Their figures, however, are based on the analysis of meat trimmed of fat and connective tissue and cannot be compared directly with those reported in this paper. The riboflavin values ranged from 2.88 to 3.9 μg. per gram of fresh veal and 2.6 to 3.0 μg. per gram of fresh lamb. These figures are somewhat higher than 2.2 μg. per gram for veal and 2.4 μg. per gram of lamb reported by Cheldelin and Williams ('42). The nicotinic acid values ranged from 49 to 84 μg. per gram of fresh veal and 48 to 68 μg. per gram of fresh lamb. These values compare favorably with 73 μg. per gram of veal and 77 μg. per gram of lamb reported by Dann and Handler ('42), and 68 to 94 μg. per gram of veal and 85 μg. per gram of lamb reported by McIntire et al., ('41).

There are interesting variations in the vitamin content of samples taken from the same carcass. This may be illustrated by values from veal samples of a single carcass. The thiamine content of the veal stewing meat, 1.43 μg. per gram, is lower than the other cuts, 1.67 μg. per gram for leg of veal and 1.7 μg. per gram of veal shoulder. The riboflavin content of the leg and sirloin of veal, 3.45 and 3.55 μg. per gram, respectively, is lower than the riboflavin values for veal shoulder and veal rib, 3.65 and 3.9 μg. per gram respectively. The opposite is true for nicotinic acid. The vitamin content of leg and sirloin cuts, both 84 μg. per gram is higher than for the shoulder cuts, 70 μg. per gram. A leg of lamb contained higher amounts of all three of the vitamins than the lamb sirloin from the same carcass. This was probably due to the difference in the fat content of the two cuts. The per cent of fat in the sirloin, 24%, was more than twice that in leg of lamb, 10%.

SUMMARY

1. Vitamin retention in veal and lamb after cooking has been studied.

2. The retention in the meat alone after roasting and broiling averaged 57 and 70% for thiamine, 82% for riboflavin and 80% for nicotinic acid.

The retention after braising of veal averaged 40% for thiamine, 73% for riboflavin and 61% for nicotinic acid. The retention after stewing averaged 26% for thiamine, 66% for riboflavin and 52% for nicotinic acid.

3. The average total thiamine retention in the meat plus drippings was 62% after roasting and braising, 80% after broiling, and only 51% after stewing. The average total retention after the various cooking methods ranged from 87 to 101% for riboflavin and 92 to 100% for nicotinic acid.

4. The vitamin content of different cuts of meats from the same carcass showed interesting variations. The average thiamine value was 1.7 μg. per gram of fresh lamb and 1.74 μg. per gram of veal. The average riboflavin value was 2.8 μg. per gram for lamb and 3.15 μg. per gram for veal, and the average nicotinic acid value for lamb was 58 μg. per gram and for veal was 67 μg. per gram.

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THE UTILIZATION OF THE SULFUR AMINO ACIDS BY THE CHICK

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ONE FIGURE

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Klose and Almquist ('41) have reported the indispensability of methionine in the diet of the chick and have shown that homocystine can replace methionine in the presence of adequate amounts of choline. In these respects the chick is similar to the rat, in which the effect of choline on homocystine utilization as a substitute for methionine was originally reported (du Vigneaud, et al., '39). Binkley and du Vigneaud ('42) have demonstrated the conversion of homocysteine to cysteine by rat liver tissue, and have shown that methionine is less effective than homocysteine in cysteine formation. This evidence has strengthened the theory of demethylation of methionine with subsequent utilization of the homocysteine residue for cysteine formation.

In the present work with chicks, a study has been made of homocystine as a substitute for cystine in the absence of dietary choline, as well as the interrelationships between cystine, homocystine and methionine in the presence of choline. S-Methylcysteine has been investigated as a choline-replacing agent for homocystine utilization and as a cystine-replacing compound. Natural and synthetic methionine have been compared for their ability to support growth.

EXPERIMENTAL

Previous studies (Klose and Almquist, '41; Almquist and Jukes, '42) of the sulfur-bearing amino acids in the diet of the chick have utilized an arachin basal diet which was deficient in methionine and several other indispensable amino acids but carried fairly large amounts of cystine. Studies on raw soybean protein as the only protein in the diet (Almquist et al., '42) have shown that the unavailability of methionine accounts almost entirely for the poor growth obtained with this protein. For this reason, an isolated soybean protein preparation was chosen for the present studies. This material was a commercial product [1] containing 86.6% protein (N $\times$ 6.25) and 1.75% ash. The protein contained 1.53 $\pm$ .08% methionine and less than .25% cystine. Methionine analyses were made according to the method of McCarthy and Sullivan ('41). Cystine was determined by precipitating with the cuprous chloride reagent of Rossouw and Wilken-Jorden ('35) and determining the amount of sulfur removed from the solution.

Preliminary experiments indicated that a basal diet in which the only protein source was the isolated soybean protein could not support a good rate of growth, but that if methionine was added, good growth was obtained. Soybean protein autoclaved for 1 hour at 15 lbs. per square inch did not lead to better growth than did the uncooked protein. This fact indicated either that the heating process did not liberate the methionine present, or that practically all the methionine present was available. In the investigations of Klose and Almquist ('41), cystine was present in adequate amount, yet the percent gain was less than zero unless methionine was added. In the present studies, the percent gain per day was 1.2 for the chicks on the basal diet without any addition of sulfur amino acids, which indicated that the amount of methionine present was largely available, although definitely suboptimal.

[1] A generous amount of this protein was supplied by the Glidden Company, through the courtesy of Mr. J. L. Gabby.

Chicks were placed on a low-choline diet at hatching time and maintained on this diet for 16 to 21 days to deplete them of choline. This diet was a modification of that used by Jukes ('41), and consisted of water-washed casein 18, glucose [2] 50.5, gelatin 8, gum arabic 5, yeast [3] 9, mineral mixture 4.24, crude soybean oil 5, and sardine oil (400 D–3000 A) 0.3 gm. per 100 gm. The mineral mixture furnished the following to each 100 gm. of diet: tricalcium phosphate 2000 mg., dipotassium phosphate 500, potassium chloride 300, sodium chloride 100, and manganese 10, silicon 46, magnesium 48, aluminum 8, iron 14, copper 1, zinc 1, iodine 0.8 and cobalt 0.5. After the depletion period, the chicks were banded, weighed and segregated into groups of three or four chicks carefully selected for weight, gain and vigor. These chicks showed a high incidence of incipient perosis, but were not yet severely crippled by the deficiency.

The basal diet to which the various supplements were added consisted of isolated soybean protein 23, glucose [2] 52.8, cellulose [4] 5, calcium gluconate 8, mineral mixture as in the low-choline diet 4.24, cottonseed oil [5] 5, cod liver oil (U.S.P.) 1, 2-methyl-1, 4-naphthohydroquinone diphosphate (sodium salt) 0.001, thiamine 0.001, riboflavin 0.001, pyridoxine 0.001, nicotinic acid 0.005, calcium pantothenate (dl) 0.003, and a biotin source [6] 1 gm. per 100 gm. The amount of soybean protein used provided 20 gm. of protein per 100 gm. of diet; hence the methionine and cystine contents of the basal diet were 0.3% and 0.05%, respectively.

The chicks were fed the experimental diets for 6 days, during which time they were weighed daily. The supplements were commercial products with the exception of S-methylcysteine, which was prepared according to the method of Clarke and Inouye ('31–'32) as modified by Brand, et al. ('37).

[2] Cerelose.

[3] Anheuser-Busch, strain G.

[4] Cellu Flour.

[5] Wesson Oil.

[6] "Molasses char," a product kindly donated by the U. S. Industrial Chemicals Company.

TABLE 1

The relation of additions of sulfur-bearing amino acids to percent gain per day.

DIET NUMBER	SUPPLEMENTS ADDED TO THE BASAL DIET [1]				TOTAL MILLIMOLS SULFUR AMINO ACIDS ADDED PER 100-GM. DIET	NUMBER OF GROUPS	AVE. % GAIN PER DAY
	l-Cystine	dl-Homocystine	dl-Methionine	Choline chloride			
1	...	...	...	...	...	2	1.2
2	0.20	...	...	...	1.67	3	1.8
3	0.40	...	...	...	3.34	2	1.9
4	0.60	...	...	...	5.01	1	2.0
5	...	0.23	...	...	1.72	1	1.8
6	...	0.45	...	...	3.36	1	1.5
7	...	...	0.25	...	1.68	1	3.7
8	...	...	0.50	...	3.36	1	4.2
9	...	...	1.00	...	6.72	1	4.3
10	0.20	0.23	...	...	3.39	2	2.0
11	0.20	0.45	...	...	5.03	1	1.6
12	0.20	...	0.50	...	5.03	1	4.4
13	...	...	...	0.20	...	1	1.1
14	0.20	...	...	0.20	1.67	2	4.0
15	0.30	...	...	0.30	2.50	1	4.0
16	...	0.23	...	0.20	1.72	2	3.8
17	...	...	0.50	0.20	3.31	1	5.3
18	0.20	0.23	...	0.20	3.39	1	5.1
19	0.20	0.45	...	0.20	5.03	2	6.4
20	0.20	...	0.25	0.20	3.35	1	5.2
21	0.20	...	0.50	0.05	5.03	1	6.0
22	0.20	...	0.50	0.20	5.03	1	6.0
23	0.20	...	0.70	0.20	6.37	1	6.3

[1] Expressed in percent added to diet.

TABLE 2

Experiments with S-methylcysteine and with l- and dl-methionine.

SUPPLEMENTS ADDED TO THE BASAL DIET [1]						TOTAL MILLIMOLS SULFUR AMINO ACIDS ADDED PER 100-GM. DIET	AVE. % GAIN PER DAY
l-Cystine	dl-Homocystine	S-Methylcysteine	dl-Methionine	l-Methionine	Choline chloride		
...	0.23	0.23	...	...	...	1.72 [2]	1.7
...	0.23	...	...	...	...	1.72	1.8
...	...	0.22	...	...	0.20	1.63	1.7
0.20	...	...	...	...	0.20	1.67	4.0 [3]
...	...	...	0.25	...	...	1.68	3.7
...	...	...	...	0.25	...	1.68	3.8
0.20	...	...	0.25	...	0.20	3.35	5.2
0.20	...	...	...	0.25	0.20	3.35	5.3

[1] Expressed in percent added to diet.

[2] On basis of homocystine alone.

[3] Mean of two groups.

DISCUSSION

The data obtained from the various experiments are presented in tables 1 and 2, which show the relationships existing between the various supplements added and the average percent gain per day. The latter quantity is calculated by the equation

$$\text{Ave. \% gain per day} = \frac{\text{Ave. gain} \times 100}{\text{Ave. wt. during expt.} \times \text{no. days on expt.}}$$

For non-depleted chicks of the same breed and of approximately the same age on a practical ration, the percent gain is about 7.5; the supplemented groups which attained the best rates of growth showed an average gain of 6.3%. This difference is not great in view of the synthetic nature of the diets.

Figure 1 has been plotted to show more clearly the relations between sulfur amino acids added and percent gain when choline is absent and present in the diet. In order to present

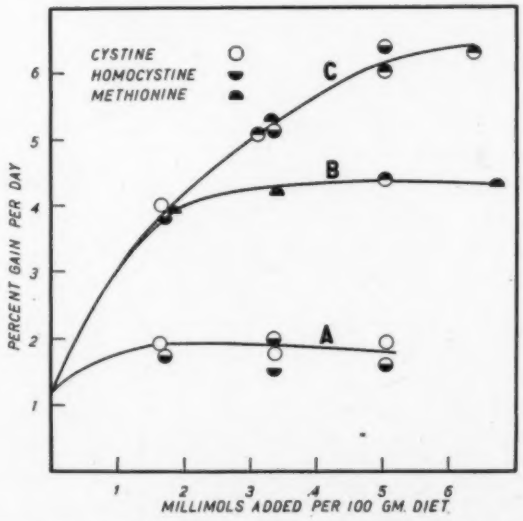

Fig. 1 The relation of added sulfur amino acids to the rate of growth. Curves A and B represented results obtained with diets to which no choline was added. The results represented by curve C were obtained with diets which contained 0.2% added choline chloride.

all data on a comparable basis, the total millimols of amino acids added have been used as abcissae. In the case of methionine, the actual number of millimols was used; with the double molecules of cystine and homocystine, twice the actual number was used.

No choline added

Up to approximately 2 millimols of added sulfur amino acids, the basal ration supplemented with methionine (fig. 1, curve B) supports a rate of growth equivalent to that shown by the groups receiving choline with homocystine or cystine (compare diet 7 with diets 14 and 16; also see fig. 1, curve C). Since the basal diet is deficient in both cystine and methionine, but contains an appreciable amount of the latter, it not surprising that, within limits, the form of amino acid added is immaterial to the growth rate. At higher amino acid levels the deficiency of choline becomes a growth-limiting influence and the percent gain increases very slowly to only 4.4 at 5 or more millimols of added sulfur amino acids, while the percent gain of the pens receiving choline in addition is about 6.3 (compare diet 12 with diets 19 and 22). These data confirm reports (Jukes, '41; Record and Bethke, '42) on the irreplaceability of choline by methionine. Curve A of figure 1 clearly shows that choline-depleted chicks cannot effectively utilize homocystine in lieu of methionine. It also indicates that homocystine is equivalent to cystine when no choline is added to the diet.

It may be merely a coincidence that the growth rates with cystine and with homocystine are practically identical; this may be the result of a partial methylation of homocysteine,[7] which is not enough to bring the gains close to those supported by methionine alone. In this event, however, it would seem that the growth effects of cystine and homocystine should be additive to some extent, which is evidently not the case. The most probable interpretation is that homocystine is converted

[7] It is assumed that the reduction of homocystine to homocysteine proceeds without interference in the choline-depleted chick. This reduction would seem to be a necessary step in the indicated conversion of homocystine to cystine, as well as to methionine.

to cystine, as indicated in the rat (Binkley and du Vigneaud, '42), and the amount of either one added is sufficient to provide all the cystine that can be utilized in the presence of the residual amount of methionine in the basal diet. This interpretation further implies that both *d* and *l* isomers of homocystine are utilized by the chick as sources of cystine when fed in a choline-deficient diet. It is realized that proof of such dual conversion is yet to be obtained.

Choline added

When choline is added to the basal diet (curve C), the same curvilinear relationship as in curve B holds between the percent gain and the added sulfur amino acids up to 2 millimols. In this range, cystine plus choline, or homocystine plus choline are equivalent to methionine without choline (compare diets 14 and 16 with diet 7).

In contrast to the curve in the absence of choline, the growth values continue to rise steeply where more than 2 millimols of sulfur amino acid are needed. Like methionine, homocystine now exerts a growth effect which is additive to that of cystine (compare diet 18 with diet 20 and diet 19 with diet 22).

S-Methylcysteine

Homocystine was fed in the absence of choline but in the presence of S-methylcysteine to demonstrate any possible methylating action of this compound (table 2). There was no difference between the group which was fed S-methylcysteine and the control group. These results are similar to those obtained with the rat (Welch, '41; Moyer and du Vigneaud, '42).

An experiment which was designed to test the cystine-replacing power of the methyl compound showed that it is ineffective in this capacity also. These results are in agreement with those obtained by Block and Jackson ('32) in experiments with the rat.

Natural and synthetic methionine

Two sets of experiments in which *l*- and *dl*-methionine were compared (table 2) showed these forms to be equally active. In the first experiment the *l* and *dl* forms were compared in the presence of 0.2% choline chloride and 0.2% cystine; in the second experiment the two forms alone were added. Both of these sets of data fit very well into the curves plotted in figure 1.

These results with chicks confirm growth experiments with rats (Jackson and Block, '33) which indicate that *d*- and *l*-methionine are equivalent. There is some discrepancy between this work and that of Bennett ('39), who reported that "*dl*-methionine is somewhat less readily utilized for growth than the *l* form." Balance experiments with dogs (Stekol, '35) disclosed no differences between the *l* and *dl* forms.

Sulfur amino acid requirements of the chick

In view of the evident equivalence of the *l* and *dl* forms, it seems permissible to conclude that former estimates of the methionine requirement of the chick, based partially on supplementation with the *dl* form, are valid. A summary of such estimates is given in table 3. These would indicate that the total cystine plus methionine requirement is approximately 1.0 to 1.1% (5.0 millimols per 100 gm.) and may be met by varying proportions of these amino acids, except that the minimum methionine level is approximately 0.5 to 0.6%.

TABLE 3

Combinations of cystine and methionine in the diet of the chick which permit maximal gains

CYSTINE	METHIONINE	SOURCE
percent	*percent*	
.25	.80	Present report
.55	.54	Almquist, et al., '42
.60	.55	Almquist and Jukes, '42
.30[1]	.60[1]	Briggs, et al., '42

[1] Possibly small additional amounts present.

SUMMARY

With the use of a diet deficient in methionine, cystine and choline, the relationships between the sulfur-bearing amino acids and choline in the chick have been further investigated. The following conclusions have been drawn:

1. The choline-depleted chick cannot appreciably utilize homocystine in place of methionine.
2. Homocystine can be utilized in lieu of cystine by choline-depleted chicks.
3. *dl*-Methionine is equivalent to *l*-methionine for growth, whether or not choline is present in the diet.
4. S-Methylcysteine can neither assist in the utilization of homocystine nor replace cystine in the diet of the chick.

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THE COMPARATIVE TOXICITY OF CALCIFEROL, A.T. 10, AND COD LIVER OIL CONCENTRATE FOR CHICKS

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In the past few years the chicken has become a popular biological tool for studying the metabolic influence of antirachitic substances. There appears, however, to be little information concerning the response of the chick to these agents if administered in massive doses.

Investigations with mammals have generally concluded that massive doses of vitamin D_2 are toxic, demonstrating increased serum calcium levels accompanied by bone dissolution. Not all reports substantiate one or the other or both of these claims. The less frequently studied irradiation product of ergosterol, dihydrotachysterol, commonly referred to as A.T. 10, has been shown (Correll and Wise, '42) to have a low antirachitic potency. The literature indicates that for mammals it is hypercalcemic and toxic. Its effect in large doses on phosphatase activity and bone structure has not been thoroughly explored. Some investigators consider this sterol a parathyroid hormone substitute and question its relation to vitamin D metabolism. Studies available on toxic levels of cod liver oil usually involve oils of relatively low vitamin D potency and stress the histological effect on certain specific organs rather than enzyme and mineral metabolism. The field of antirachitic sterol metabolism has been admirably reviewed by Reed, Struck, and Steck ('39). McLean ('41) has published a later survey concerning A.T. 10.

It seemed likely that the use of the chick in studies involving massive doses of these antirachitic agents might add to our knowledge concerning their mode of action. By following the serum calcium, phosphorus and phosphatase values as well as bone ash content in the experiments herein reported, we hoped to learn in what ranges vitamin D_2, A.T. 10 and vitamin D from cod liver oil were toxic for chicks and if in massive doses they would produce hypercalcemia with bone dissolution.

The data were also expected to reveal whether A.T. 10 would augment serum phosphatase levels in the chick, as parathyroid hormone reportedly does in mammals (Cantarow, Brundage and Housel, '37; Gutman, Tyson and Gutman, '36), or whether it would, like vitamin D, maintain low normal concentrations.

Finally, it has been our experience as previously reported (Correll and Wise, '38) that rachitic birds with characteristic low bone ash demonstrate a greatly elevated serum phosphatase concentration. If massive doses of any of these substances resulted in bone demineralization as indicated by low bone ash, we were anxious to ascertain whether serum phosphatase values would remain low or would rise as they always do in rachitic subjects with faulty bone composition. The answer obtained might be helpful in the solution of the problem as to whether the greatly increased phosphatase content of plasma in rickets and certain bone diseases is the cause or effect of the recognized mineral insufficiency.

EXPERIMENTAL

Single comb white Leghorn chickens when 1 day old were divided into groups of twenty and placed on a rickets-producing basal ration consisting of ground yellow maize 57 parts, wheat flour middlings 25, crude casein 12, calcium carbonate 1, calcium phosphate 1, iodized salt 1, brewer's yeast 2, and maize oil 1. Analysis revealed the mixture to contain 1.0% calcium and 0.7% phosphorus. Some assay groups at the higher levels, as indicated in table 1, included fewer birds

because the quantity of high potency material available was limited.

The sterols used were so diluted when possible (exceptions are noted in the table) that 0.1 ml. contained the desired daily dose. This was administered to each chick per os with a 1-ml. tuberculin syringe carrying an 18-gauge blunted needle. Injections were made daily, except Sunday, over a period of 21 days at which time those that survived were sacrificed. Material for analysis was taken from each remaining bird up to fifteen from a group. The technique for obtaining pooled blood and bone samples and determining bone ash was essentially as described in a prior report (Correll and Wise, '38). Serum calcium values were ascertained by the method of Clark and Collip ('25), phosphorus and phosphatase according to Bodansky ('33). Judgment as to toxic doses was based on the gross appearance of the birds, failure to grow as shown by weekly weighings, and the number of chicks that died towards the end of the experimental period.

The vitamin D unitage, as indicated by U.S.P. XII rat assays conducted in this laboratory, served as a basis for deciding at what dosages the substances under investigation were administered to the chicks. The lower levels of vitamin D from cod liver oil were prepared from an oil having a potency of 270 I.U. per gram; the massive doses from a concentrate assaying 66,000 I.U. per gram. The smaller doses of vitamin D_2 were dilutions of a solution of irradiated ergosterol with an activity of 10,000 I.U. per gram; massive doses were made up from crystalline calciferol (m.p. 116°C.) assaying 40,000,000 I.U. per gram. The dihydrotachysterol dilutions originated from a commercial product [1] which showed an antirachitic potency of 30,000 I.U. per gram of sterol content.

Approximately ten separate experiments were conducted. Each consisted of ten groups including a negative and positive control group. The negative controls received only the basal ration. As positive controls we used chicks that were fed ad

[1] Hytakerol. Winthrop Chemical Company, New York.

lib. throughout the experimental period the basal diet in which had been incorporated into the maize oil portion sufficient cod liver oil to furnish 20 I.U. of vitamin D in each 100 gm. of ration. Previous feeding experiments have shown us that such a supplement in the basal diet is about minimal for normal mineral, phosphatase, and bone ash response in chicks of this age and hence could be used as a standard of comparison to determine the adequacy of an antirachitic substance administered at different levels and by any route. Past records of food consumption on groups similar to these positive controls were only obtained for the group as a whole but they allowed an estimation of the daily intake of vitamin D per chick to be around 2 to 3 I.U. This estimation served as a basis for establishing more accurately the individual daily minimum requirement.

RESULTS AND DISCUSSION

Since the minimum daily prophylactic dose of the sterols to be studied necessary to maintain normal serum calcium, phosphorus, and phosphatase values, and bone ash content in chicks, was not known, our first objective was to establish these values. We then proceeded to determine toxic levels and observe the response to toxic and subtoxic doses.

The data obtained are presented in condensed form in table 1. The values recorded for the controls are averages for such groups from all the experiments. The physiological effects on the chick of the sterols listed were studied several times at numerous levels. The groups submitted in table 1 for comparison with the control values represent typical responses to the doses indicated.

It can be seen in experiment 1 that normal serum calcium, phosphorus, and phosphatase values, and adequate bone ossification, were attained upon the daily administration of 3 I.U. of vitamin D from cod liver oil (group 555). Toxicity was evident at a level around 15,000 I.U. of vitamin D (group 556) from this particular concentrate, or at 5000 times the minimum daily dose. Even the massive doses produced no hypercalcemic

TABLE 1

Serum calcium, phosphorus, and phosphatase (per 100 ml. of serum), and bone ash values of chicks given massive doses of several sterols.

GROUP NO.	DAILY DOSE Weight	DAILY DOSE I. U.	FINAL WEIGHT	INORGANIC CALCIUM	INORGANIC PHOSPHORUS	PHOSPHATASE	BONE ASH	COMMENTS
			gm.	*mg.*	*mg.*	*units* [2]	%	
				Controls				
Negative	0	.....	133	6.8	5.5	131	36.2	
Positive	20 I.U./100 gm.[1]	.....	152	10.4	5.9	44	47.0	
				Experiment 1. Vitamin D from cod liver oil				
501	3.7 mg.	1	149	7.9	5.5	103	39.9	
555	11.1 mg.	3	148	10.0	6.0	38	47.1	
506	15.2 mg.	1000	151	10.5	5.8	38	48.0	
508	152 mg. 0.2 ml.	10,000	142	10.2	5.4	35	49.0	
556	228 mg. 0.3 ml.	15,000	109	10.1	5.9	27	50.0	Two birds died. Toxic level.
558	380 mg. 0.5 ml.	25,000	91	10.3	5.5	28	49.0	Ten birds died.
				Experiment 2. Calciferol				
477	0.25 μg.	10	144	8.3	4.6	90	40.0	
536	2.5 μg.	100	142	10.5	5.3	47	46.9	
580	25 μg.	1,000	136	10.7	8.4	54	47.3	
582	0.25 mg.	10,000	137	11.6	8.2	33	49.0	
544	2.5 mg.	100,000	137	13.0	4.2	29	44.5	Five birds; all lived.
573	3.1 mg.	125,000	...	...	..	...	...	Five birds; four died. Toxic level.
				Experiment 3. Dihydrotachysterol (A.T. 10)				
494	8.3 μg.	0.25	104	8.9	5.5	75	39.2	
546	16.6 μg.	0.5	154	10.6	5.0	44	49.7	
547	33.3 μg.	1	147	11.2	6.0	33	48.3	
548	66.6 μg.	2	160	12.4	4.8	45	47.2	
562	166.5 μg. 0.2 ml.	5	94	13.8	4.5	29	43.7	Ten birds; six died. Toxic level.
563	249.8 μg. 0.2 ml.	7.5	63	14.5	..	< 27	38.6	Eleven birds; nine died.

[1] For explanation see text under Experimental.

[2] Bodansky, '33.

effect nor bone dissolution. Antithetically, as the dose tended to be toxic, bone calcification if anything became more dense (groups 556, 558). Serum phosphatase concentrations remained within the normal range or lower.

The toxicity of such a concentrate for chicks, when given at levels of better than 200 mg. per day, may well be due to some constituent unrelated to the antirachitic entity. However, the data herein presented show that extremely high doses of vitamin D from cod liver oil in the chick did not cause a deviation from normal of the serum calcium, phosphorus or phosphatase levels, or bone ash content.

In experiment 2 the daily administration of 100 I.U. of vitamin D_2 (group 536), yielded mineral, enzyme and bone values comparable to the positive controls. Doses around 100,000 I.U. per day (group 544), or 1,000 times the minimum requirement, approached a toxic range. With calciferol in this quantity of 2.5 mg. daily, representing the enormous dose of 100,000 I.U. of vitamin D_2, there was a slight rise in serum calcium and indications that the mineral content of the bone was somewhat depleted, but the serum phosphatase remained low. Small additional increases in the daily dose (group 573) were so toxic as to be fatal for the birds before the end of the experimental period.

A.T. 10 (experiment 3) demonstrated normal responses when 0.5 I.U. were given daily (group 546), and toxic manifestations at ten times this level or 5 I.U. per day (groups 562). The fact that 16.6 μg., equivalent to only 0.5 I.U. of antirachitic activity, was sufficient to yield mineral responses in the chick comparable to those obtained by 3 I.U. from cod liver oil (group 555) or 100 I.U. of vitamin D_2 (group 536) again emphasizes (Correll and Wise, '42) the remarkable difference in the antirachitic substance present in A.T. 10.

As the daily level of A.T. 10 was increased towards the definitely toxic doses (groups 562, 563) the serum calcium became progressively higher while bone ash values receded. It will be noted, however, that with the obvious bone dissolu-

tion, the serum phosphatase concentrations never rose; in fact, they actually went below the positive controls.

Thus in the chick this interesting sterol of plant origin demonstrates a greater antirachitic efficiency on a rat unit basis than the vitamin D from cod liver oil. In contrast, however, its toxic dose is only about ten times its minimum protective level and this is accompanied by a pronounced increase in serum calcium concentration with bone decalcification. The hypercalcemia resembles the action of parathyroid hormone but no increase was found in the serum phosphatase as has been reported for parathyroid hormone in other species.

In no instance, where there was bone dissolution after administration of massive doses of an antirachitic agent, was there any rise in serum phosphatase. This suggests that the increased enzyme values observed in rickets is probably not a direct result of a physical weakening of the bone structure per se.

SUMMARY

1. Vitamin D from cod liver oil at 3 I.U. (11.1 mg.) daily affected normal mineral responses in chicks. When given at high levels no deviation from normal was seen with this particular concentrate, in the serum calcium, phosphorus, and phosphatase or the bone ash values; toxic manifestations were noted at about 5,000 times the minimal dose. It is recognized that the observed toxicity for the concentrate may be due to something besides its vitamin D activity.

2. Calciferol at 100 I.U. (2.5 μg.) yielded normal values; toxicity was apparent at about 1,000 times this dose. In enormous doses a slight hypercalcemia and bone dissolution was found. Phosphatase values remained low at these high levels.

3. Only 0.5 I.U. (16.6 μg.) of antirachitic activity was required daily from A.T. 10 to produce normal mineral metabolism in the chick. This emphasizes the individuality of the antirachitic substance present in A.T. 10 when compared with the other rickets-preventing sterols studied. A.T. 10 was

toxic for chicks in these experiments at levels around ten times the minimal daily requirement. In the massive doses there was a hypercalcemia and bone dissolution.

4. A.T. 10, like vitamin D, will prevent rickets in chicks. Its effect in increasing serum calcium and lowering bone ash appears to be of a degree that resembles parathyroid hormone. However, even in massive doses it caused no increase in serum phosphatase. Thus this sterol preparation influences calcium metabolism in a manner which resembles both vitamin D and parathyroid hormone.

5. In no case where massive doses of sterols resulted in bone ash depletion, was there observed any increase in serum phosphatase concentration.

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CAROTENE REQUIREMENTS FOR THE MAINTENANCE OF A NORMAL SPINAL FLUID PRESSURE IN DAIRY CALVES[1]

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The minimum requirement of calves for carotene has been the subject of several recent reports. Guilbert and co-workers ('37) reported a minimum requirement for growing calves of 30 μg. of carotene per kilogram of body weight using nyctalopia as a criterion. This requirement they believed to be the physiological minimum. Moore ('39c) confirmed the figure given by Guilbert using nyctalopia as a criterion but stated that this amount was not sufficient to prevent papilledema in calves. Therefore, the 30 μg. level would be below the physiological minimum. Boyer, Phillips, and co-workers ('42a) found 75 μg. per kilogram for Holstein yearlings and 125 μg. per kilogram for Guernsey yearlings necessary to maintain an adequate plasma vitamin A level. Keener and associates ('42) found that 12 μg. per pound (27 per kilogram) met the minimum requirements at an environmental temperature of 50° to 70°F. However, these investigators state that during severe winter weather the minimum requirement may be more than twice as great as during warm weather. Ophthalmoscopic observations, blood vitamin A levels, and histopathological alterations were used as criteria.

[1] Scientific Paper no. A57, Contribution no. 1893, of the Maryland Agricultural Experiment Station. (Department of Dairy Husbandry.) Journal Article no. 651. n.s. of the Michigan Agricultural Experiment Station.

Moore ('39b), Wetzel and Moore ('40) reported papilledema in calves fed vitamin A deficient rations. Also, it was demonstrated that the papilledema could be prevented by feeding crystalline carotene dissolved in cottonseed oil. Papilledema is a result of an increase in pressure of the cerebrospinal fluid. Moore and Sykes ('40) ('41) found abnormally high pressures of the spinal fluid in calves fed vitamin A deficient rations which could be alleviated by feeding crystalline carotene dissolved in cottonseed oil.

Because of these results it was believed that periodic measurements of the pressure of the spinal fluid might be used as a criterion for the adequacy or inadequacy of various intakes of carotene. Data collected from such experiments are herein reported.

EXPERIMENTAL

Two groups of calves were used in this experiment. The data on the first group were obtained at the dairy section of the Michigan Agricultural Experiment Station, East Lansing, while the data on the second group were collected at the dairy section of the Maryland Agricultural Experiment Station, College Park. Pertinent differences in the management, experimental procedures, and results will be alluded to throughout the paper.

Calves 3 months of age were placed on a basal ration low in carotene for a 30-day depletion period, after which carotene in the form of dehydrated alfalfa leaf meal was added at various levels of intake. The basal ration consisted of 240 lbs. of ground barley, 180 lbs. of rolled oats, 180 lbs. of wheat bran, 60 lbs. of linseed meal, and 8 lbs. of salt. Ten pounds of skimmed milk per calf was fed to group I while 1 lb. of skimmed milk powder was fed daily to group II. Viosterol was fed as a source of vitamin D during winter months. Wood shavings were used as bedding.

The calves in group I were weighed every 10 days and those in group II, weekly, at which time adjustments were

made on the intake of carotene which was fed according to body weight. The small amount of carotene in the basal ration was taken into account.

The carotene content of the alfalfa leaf meal was determined chromatographically by a previous published procedure (Moore, '40 and Moore and Ely, '41). Weekly carotene determinations on the blood plasma of the first group were made by a previously published method (Moore, '39a). Plasma vitamin A and carotene on the second group were determined by a modification of the previous carotene method as suggested by Kimble ('39). The determinations were made with a photoelectric spectrophotometer with a slit width of 5 millimicrons. A wave length of 455 millimicrons was used for carotene and 620 for the antimony trichloride reaction for vitamin A. Plasma ascorbic acid determinations for group II were made every 2 weeks by the macro method of Mindlin and Butler ('38). A wave length of 510 millimicrons was used to determine the fading of the dye.

The method for making the spinal puncture and the measurement of the spinal fluid pressure has previously been described by Sykes and Moore ('42). In the second group of calves a small bore glass tube replaced the water manometer which obviated releveling with changes in the position of the head or body of the calves. The glass tube was connected to the needle by means of a short piece of rubber tubing and the height to which the spinal fluid rose in the tube was noted. Ophthalmoscopic observations were made periodically.

RESULTS AND DISCUSSION

Tables 1 and 2 show the results obtained with the two groups of calves. These data have been summarized for 30-day periods. Since samples of blood for vitamin A and carotene determinations were drawn each week, the values shown are the average of two to five determinations. The plasma ascorbic acid values are the average of two determinations.

TABLE 1

Results in 30-day periods with Ayrshire and Holstein calves in group I.

	INTAKE 46 μG. CAROTENE PER KG. C 440 AYR. MALE		INTAKE 62 μG. CAROTENE PER KG. C 442 HOL. MALE		INTAKE 62 μG. CAROTENE PER KG. C 439 AYR. MALE		INTAKE 68 μG. CAROTENE PER KG. C 468 HOL. MALE		INTAKE 77 μG. CAROTENE PER KG. C 456 HOL. MALE		INTAKE 77 μG. CAROTENE PER KG. C 457 HOL. MALE		INTAKE 123 μG. CAROTENE PER KG. C 441 HOL. MALE	
	12-5-39 [1]		2 10-40 [1]		12-5-39 [1]		5-26-41 [1]		11-8-40 [1]		11-27-40 [1]		12-5-40 [1]	
AGE	Plasma carotene	Spinal fluid pressure	Plasma carotene	Spinal fluid pressure	Plasma carotene	Spinal fluid pressure	Plasma carotene	Spinal fluid pressure	Plasma carotene	Spinal fluid pressure	Plasma carotene	Spinal fluid pressure	Plasma carotene	Spinal fluid pressure
Days	*μg. per 100 ml.*	*m. m. H O*	*μg. per 100 ml.*	*m. m. H O*	*μg. per 100 ml.*	*m. m. H O*	*μg. per 100 ml.*	*m. m. H_2O*	*μg. per 100 ml.*	*m. m. H O*	*μg. per 100 ml.*	*m. m. H O*	*μg. per 100 ml.*	*m. m. H_2O*
120–150	19	105	16	110	12	90	...	90	37	80	31	80	30	120
150–180	37	115	21	120	27	125	...	...	35	95	42	85	59	...
180–210	45	120	30	160	43	110	...	130	43	90	41	90	85	105
210–240	50	165	35	200	66	135	...	...	41	110	43	95	83	85
240–270	59	170	40	190	98	...	93	120	48	85	40	90	78	95
270–300	73	165	58	240	92	...	80	115	42	90	27	120	83	...
300–330	47	200	40	200	87	...	...	...	26	105	39	100	71	120
330–360	75	165	32	...	80	160	...	...	36	105	54	...	90	110
360–390	41	...	32	210	53	...	121	120	82	...	..	90	90	...
390–420	59	210	35	180	72	190	82	120	..	90	..	...	82	120
420–450	84	220	..	...	93	190								
450–480	..	...	..	...	100	190								

[1] Date when 120 days of age.

TABLE 2

Spinal fluid pressure and plasma vitamin A, carotene and ascorbic acid in calves of group II in 30-day periods

DATE	AGE	PLASMA			SPINAL FLUID PRESSURE
		Vitamin A	Carotene	Ascorbic acid	
	days	*μg./100 ml.*			mm. H_2O
Intake 44 μg. carotene/kg.					
474 Holstein Male					
7- 5-42	120	2	4	..	...
8- 4-42	150	5	8	.18	85
9- 3-42	180	8	17	.30	75
10- 3-42	210	8	18	.36	75
11- 2-42	240	7	24	.27	125
12- 2-42	270	7	29	.32	130
1- 1-43	300	7	18	.28	180
1-30-43	330	7	22	.17	210
3- 1-43	360	.	..	..	...
Intake 62 μg. carotene/kg.					
463 Ayrshire Male					
2-16-42	120	12	6	..	95
3-18-42	150	13	36	..	...
4-17-42	180	14	51	..	130
5-17-42	210	14	46	.34	110
6-16-42	240	14	38	.29	85
7-16-42	270	15	38	.27	85
8-15-42	300	15	47	.27	75
9-14-42	330	12	45	.31	90
10-14-42	360	12	55	.25	120
11-13-42	390	14	34	.18	...
12-13-42	420	12	47	.29	155
1-12-43	450	11	49	..	195
470 Holstein Female					
6-15-42	120	15	24	.33	70
7-14-42	150	16	36	.29	70
8-13-42	180	14	57	.32	70
9-12-42	210	14	52	.28	110
10-12-42	240	11	53	.25	115
11-11-42	270	12	51	.30	140
12-11-42	300	9	47	.31	170
1-10-43	330	8	56	.23	230
Intake 70 μg. carotene/kg.					
466 Holstein Male					
4- 4-42	120	10	25	..	85
5- 4-42	150	9	34	.25	...
6- 3-42	180	10	39	.27	70
7- 3-42	210	8	24	.26	...
8- 2-42	240	11	30	.31	70
9- 1-42	270	10	38	.37	70
10- 1-42	300	7	59	.31	70
10-30-42	330	8	60	.35	80
11-29-42	360	8	43	.35	95
12-29-42	390	7	52	.32	85

DATE	AGE	PLASMA			SPINAL FLUID PRESSURE	
		Vitamin A	Carotene	Ascorbic acid		
	days	*μg./100 ml.*			mm. H_2O	
Intake 62 μg. carotene/kg.						
472 Guernsey Male						
6-28-42	120	9	24	..	...	
7-28-42	150	13	38	.34	115	
8-27-42	180	10	49	.31	75	
9-26-42	210	7	36	.28	120	
10-26-42	240	10	32	.33	...	
11-25-42	270	10	36	.31	160	
12-25-42	300	8	33	.20	190	
1-24-43	330	6	39	..	160	
Intake 66 μg. carotene/kg.						
464 Ayrshire Male						
4-12-42	120	14	40	..	65	
5-12-42	150	15	33	.28	65	
6-11-42	180	13	41	.28	70	
7-11-42	210	14	40	.30	...	
8-10-42	240	12	63	.34	70	
9- 9-42	270	10	46	.26	80	
10- 8-42	300	10	47	.50	75	
11- 7-42	330	11	49	.25	105	
12- 7-42	360	8	37	.27	115	
1- 6-43	390	11	59	.23	85	
2- 5-43	420	11	67	..	...	
471 Ayrshire Male						
6-15-42	120	7	11	.27	70	
7-15-42	150	6	7	.27	75	
8-14-42	180	7	11	.20	80	
9-13-42	210	9	10	.24	75	
10-13-42	240	11	20	.38	70	
11-12-42	270	7	34	.30	100	
12-12-42	300	7	34	.27	100	
1-11-43	330	5	30	.16	95	
Intake 75 μg. carotene/kg.						
467 Holstein, Female						
5-17-42	120	8	20	..	...	
6-16-42	150	11	27	.23	65	
7-16-42	180	11	31	.29	65	
8-15-42	210	8	34	.23	115	
9-14-42	240	9	27	.20	85	
10-14-42	270	11	52	.22	75	
11-13-42	300	10	47	.36	145	(excited)
12-13-42	330	7	55	.22	115	
1-12-43	360	7	66	.28	110	

The results show that an intake of 62 μg. of carotene per kilogram was not sufficient to prevent an increased spinal fluid pressure. Animals C442 and C439 of group I and 463, 470, and 472 of group II demonstrate this fact. On the other hand, an intake of 66–68 μg. was sufficient to maintain a normal spinal fluid pressure as shown by animals C468 of group I and 464 and 471 of group II. It would seem, therefore, that the measurement of the spinal fluid pressure is an exceedingly critical measure for detecting small differences in carotene intake. It appears that the minimum requirement is about 66 μg. per kilogram of body weight. These requirements are a little more than double those reported by Guilbert and associates ('37) and confirmed by Moore ('39c) where nyctalopia was used as a criterion. The differences noted are probably due to the different criteria used in this study as compared to the former studies, although environmental factors may be partly responsible. Moore ('39c) has shown previously that papilledema and presumably increased spinal fluid pressure developed in calves in which nyctalopia was not present.

It should be kept in mind that these requirements are for Holstein and Ayrshire calves from 4 months to 12 to 14 months of age. It is possible that calves from birth to 4 months of age have a higher requirement, and experiments reported by Converse and Meigs ('39) indicate that this may be true. The work by Kuhlman and Gallup ('40) shows a requirement of 40 μg. per pound (88 per kilogram) is necessary for proper reproduction.

The results further show the calves in group I receiving 62 μg. or less developed pressure during the early spring months so that they went into the summer months with high pressures. The pressures showed no tendency to decrease during the summer months. On the other hand, the calves in group II receiving 62 μg. or less did not develop the increased spinal fluid pressure during the summer months. The increase in pressure was delayed until the winter months. These results might at first glance appear contradictory unless certain facts of climate and management are taken into con-

sideration. For instance the average July temperature at East Lansing, Michigan, is 71.1°F., while that for College Park, Maryland, is 75.9°F., (Yearbook, '41). Furthermore, the humidity during the summer months at East Lansing is lower than at College Park making the effective temperature lower. A possible further contributing factor is the fact that at East Lansing the calves of group I were always placed in the barn by 10:30 A. M. or before, while at College Park the calves in group II were left to considerable exposure to sunshine throughout the day. Under these conditions high body temperatures, 104–105°F. were often recorded for calves in group II in the summer months.

It is not possible with the present data to compare the requirements of the calves at East Lansing during the summer months with those of the calves at College Park for a similar period nor is it possible to definitely ascribe the differences noted above to environmental temperature. However, these data do suggest that where spinal fluid pressure is used as a criterion, the requirement is higher during the winter months. The data are somewhat more conclusive in that they tend to confirm the excellent observations of Keener and associates ('42). While Keener did not make spinal fluid pressure measurements, he did make ophthalmoscopic observations for papilledema which is a result of an increased spinal fluid pressure. Keener also observed lower plasma vitamin A values during the winter months. The results in these experiments confirm that observation.

A study of the plasma vitamin A values does not show any close correlation between carotene intake and these plasma vitamin A values except where wide variations of intake are considered. As a matter of fact the vitamin A values for calves 470 and 463 receiving an intake of 62 μg. of carotene are higher than for calves 471 receiving 66 μg., 466 receiving 72 μg., and 467 receiving a 75 μg. intake. Within these narrow limits of intake therefore vitamin A values of the blood plasma are of little value as a criterion of adequacy or inadequacy. This fact seems to be fairly well established since these calves

were maintained on the same level of intake over a period of 10 to 12 months with blood samples being drawn every week. There would seem to be individual characteristics of each animal which determine the level of vitamin A in the blood. A study of the data presented by Davis and Madsen ('41) appears to indicate considerable variation. The same conclusion can be drawn from a study of the carotene values. It should be emphasized that at times there are considerable weekly and periodic variations in the vitamin A and carotene values with these levels of intake. When the intakes are cut down to 16 μg. or below, the carotene variations are much less pronounced as shown by previously published data (Moore, '39c).

It is not the purpose of this discussion to leave the impression that determinations of plasma vitamin A values are of no value. From a practical standpoint, if the limitations are understood, they are quite valuable. The values for animal 474 receiving only 44 μg. are apparently lower than for the other animals. Therefore, one could probably tell the difference between an intake of 44 and 75 μg. but not between 62 and 75.

Among the seven calves which received less than 66 μg. of carotene per kilogram of body weight and which showed an increased spinal fluid pressure, four developed papilledema. The nerve heads of the other three calves apparently remained normal. Two factors working together probably account for this variation. First, most of these seven calves received carotene just below the minimum rate so that a marked increase in spinal pressure did not develop. The higher the spinal pressure the more marked is the papilledema. Secondly, there are some age and individual variations in cattle of the intraocular tension (unpublished data). A high intraocular tension would prevent the papilledema from developing especially where the spinal fluid pressure was not greatly elevated.

Boyer and co-workers ('42b) have pointed out a possible relationship between vitamin A and C and the pressure of the spinal fluid. Consequently plasma ascorbic acid determinations

were made on the calves of group II. No particular correlation between plasma ascorbic acid levels and increased spinal fluid pressure is noted. However, since the pressures are not high and since the variations of carotene intake are small, the authors wish to withhold further comment until more critical experiments now in progress have been completed.

SUMMARY

1. The results of these experiments indicate that measurement of the spinal fluid pressure of calves is a fairly critical index of adequacy or inadequacy of carotene intake.

2. An intake of 66 μg. per kilogram of body weight during the winter months is about the minimum requirement for carotene for Holstein and Ayrshire calves when spinal fluid pressure is used as a criterion.

3. Because of individual characteristics, plasma vitamin A or carotene values will not distinguish between variations of carotene intake of 62 to 75 μg. per kilogram of body weight.

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INFLUENCE OF INCREASING DOSES OF THIAMINE AND RIBOFLAVIN ON EFFICIENCY OF THEIR UTILIZATION [1]

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Knowledge of the relation of size of dose to efficiency of utilization of the B vitamins and what happens to excessive doses is important for many reasons. It should throw some light on the question of the true physiological minimum. It should also indicate whether the body's disposition of excessive doses involves merely the excretion of amounts that are greater than can be stored, or whether this excess is actually destroyed by the tissues, a fact that would serve to explain in part at least why some patients have been found to require unusually large amounts of thiamine in order to show clinical improvement.

A review of the literature disclosed that Leong ('37) made a study of the metabolism of vitamin B_1 and investigated the maximum storage of that vitamin in rats. He used the bradycardia method of Drury and associates ('30), which, compared with chemical procedures now available, must be considered qualitative rather than quantitative. This investigator concluded that the fecal excretion of vitamin B_1 in rats, apart from the small constant output attributable to bacterial synthesis, was insignificant with intakes of less than 150 μg. Incomplete absorption of the vitamin occurred when this level of intake was exceeded. When 750 μg. of vitamin B_1 were injected sub-

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cutaneously, 75% of this dose was excreted in the urine. Maximum storage was obtained when the intake of vitamin B_1 was 90 μg. per day, a further increase above this level giving rise to no appreciable accumulation of tissue reserves.

The qualitative nature of Leong's procedures and the use of a crude product of thiamine invited a further investigation of this problem, using quantitative chemical methods and pure crystalline riboflavin as well as thiamine.

In a recent communication (Sure and Ford, '42) dealing with thiamine and riboflavin interrelationships in metabolism, a technique was described for determining these vitamins in rat urine and feces, using fluorophotometric procedures. For thiamine we employed the method of Hennessy and Cerecedo ('39), as modified by the research laboratories of Merck and Company ('41). For the riboflavin determination in feces, tissues, and glands we followed the method of Conner and Straub ('41). For the riboflavin analysis of urine we used our modification of the method of Hodson and Norris ('39) for determining the riboflavin content of foodstuffs, the details of which are described elsewhere (Sure and Ford, '42).

The early results obtained on the riboflavin content of rat feces were very erratic, the weekly fecal excretion frequently being twice or more than the total intake. The high figures were most probably due to bacterial synthesis (Wildemann, '41; Mitchell and Isbell, '42). Bacterial synthesis in the rat feces were finally reduced to much smaller proportions by collecting the feces under petroleum ether, the solvent used in the next step for removing fats previous to analysis. On the whole, however, the quantitative chemical methods for the determination of thiamine and riboflavin in rat urine and feces seemed satisfactory enough to warrant efforts being made to obtain balance sheets on the intake and output of graduated increased doses from small to massive amounts, in order to determine the efficiency of utilization of these vitamins in the animal organism. The results of this study are submitted in tables 1 to 6 inclusive.

EXPERIMENTAL

Thiamine and riboflavin balance experiments were carried out on twenty-four male albino rats, 36 days of age, and weighing from 80 to 100 gm. They were fed ad libitum a purified ration of the following composition: Casein,[2] 18; agar-agar, 2; Sure's salts no. 1 (Sure, '41), 4; butter fat, 10; dextrose, 66. This diet was supplemented by daily allowances of 20 μg. pyridoxine, 6 mg. choline chloride, and 200 μg. calcium pantothenate. The thiamine and riboflavin were administered in daily doses of 5, 10, 20, 50, 100, and 1,000 μg. The metabolism experiments were carried out in groups of four. In each group two animals received the same thiamine and riboflavin doses, one was denied thiamine, and another was not given any riboflavin. Each group, therefore, had a negative thiamine and a negative riboflavin control, which furnished information on the amounts of these vitamins contributed by the body tissues in the urinary and fecal excretions. The first three groups were given daily doses of 5, 100, and 1,000 μg. and the metabolism experiments were conducted for 26 days. However, since it was found that one-half that period provided sufficient evidence for calculation on efficiency of utilization, the balance studies on 10, 20, and 50 μg. were continued for only 13 days.[3] The thiamine and riboflavin determinations in the urine were carried out 5 days a week. When 48-hour samples were run the urine was stored in a refrigerator, preserved with toluol and kept acid at a pH of 4 to 4.5. The feces, collected in bottles covered with petroleum ether, were analyzed once weekly. The urine of the animals receiving the high doses had to be considerably diluted, so that the readings in the fluorophotometer were approximately 1 μg. Of course, the negative controls excreted very small amounts of both thiamine and riboflavin, in many in-

[2] Secured from the Borden Company, New York under the trade name "Labco".

[3] When these studies were begun we still experienced considerable difficulties with bacterial synthesis of fecal riboflavin; hence, it was necessary to discard 1 or 2 weeks' metabolism data, and for this reason the periods of experimentation in the riboflavin studies were less than in the thiamine investigations. However, 2 to 3 weeks' balance studies yielded sufficient information to warrant the conclusions, presented in this paper.

stances, only tenths of a microgram. The change in body weights and food consumption during the experimental periods are presented in table 3. It will be noted that on the daily doses of 10, 20, and 50 μg. of thiamine and riboflavin there were only slight differences in gains of body weight; also, very small differences in increases of weight were apparent on the 100- and 1,000-μg. daily doses. The differences in efficiency of thiamine utilization, however, varied considerably with daily dose.

Thiamine utilization

Allowing for the thiamine excreted on a thiamine-deficient ration, the fecal excretions on the 5-, 10-, 20-, 50-, 100-, and 1-000-μg. daily doses represented 8.9, 5.0, 19.8, 28.9, 21.1, and 27.8% of the total daily intake, respectively. The urinary excretions of thiamine on the same increasing daily doses constituted 5.2, 3.7, 5.5, 5.6, 13.2, and 34.8%, respectively of the total intake. The thiamine absorbed is considered to be equal to the total intake minus the total excreted in the feces, corrected for the amounts excreted on a thiamine-deficient ration. The thiamine retained is regarded as equal to the thiamine absorbed less that excreted in the urine on a thiamine-deficient ration. The per cent thiamine utilized, then, equals

$$\frac{\text{thiamine retained}}{\text{thiamine absorbed}} \times 100$$

The riboflavin figures were calculated in similar fashion.

It is evident from table 1 that the fecal excretion of thiamine as per cent of the total intake rises with the increase of the dose, beginning with the 20-μg. daily dose; also, the rise in the urinary excretion of thiamine as per cent of the total intake does not begin until the 100-μg. dose. The 5, 10, 20, and 50-μg. daily doses were most efficiently utilized. Beginning with the 100-μg. dose, the efficiency of utilization begins to fall, and drops markedly on the 1,000-μg. dose to 51.9%. On the latter high dose of thiamine intake there is very poor retention, as evidenced by large proportions of urinary excretions of this vitamin. There is very little difference, however, in the ab-

TABLE 1

Influence of increasing doses of thiamine on efficiency of their utilization.

ANIMAL NUMBER ♂	METABOLISM PERIOD	DAILY THIAMINE INTAKE	TOTAL THIAMINE INTAKE	THIAMINE EXCRETED IN FECES [1]	THIAMINE ABSORBED		THIAMINE EXCRETED IN URINE [1]	THIAMINE RETAINED	THIAMINE UTILIZED	TOTAL EXCRETIONS OF THIAMINE IN FECES AND URINE	
	days	*μg.*	*μg.*	*μg.*	*μg.*	*μg.*	*μg.*	*μg.*	%	*μg.*	%
1	26	5	130	10.2	119.8	92.1	7.3	112.5	93.9	17.5	13.4
2	26	5	130	12.8	117.2	90.1	6.2	111.0	94.9	19.0	14.6
3	13	10	130	6.1	123.9	95.3	5.2	118.7	95.8	11.3	8.7
4	13	10	130	6.9	123.1	94.7	3.5	119.6	97.2	10.4	8.0
5	13	20	260	47.9	212.1	81.6	13.6	198.5	93.6	61.5	23.5
6	13	20	260	55.0	205.0	78.8	15.0	190.0	92.7	70.0	26.9
7	13	50	650	190.8	459.2	70.6	37.8	421.4	91.6	228.6	35.2
8	13	50	650	185.3	464.7	71.5	34.4	430.3	92.6	219.7	33.8
9	26	100	2,600	549.3	2,050.7	78.9	338.1	1,712.6	83.5	887.4	34.1
10	26	100	2,600	574.8	2,025.2	77.9	347.9	1,677.3	82.3	922.7	35.5
11	26	1,000	26,000	7,087.6	18,912.4	72.7	8,544.4	10,368.0	54.8	15,632.0	60.1
12	26	1,000	26,000	7,353.6	18,646.4	71.5	9,508.4	9,138.0	49.0	16,862.0	64.9

[1] Corrected for the amount excreted on a thiamine-deficient ration.

sorption of thiamine on the 50-, 100-, and 1,000-μg. doses. The increased losses of thiamine in both feces and urine begin with the 20-μg. dose from 25% on this level of intake to 63% on the 1,000-μg. dose.

Riboflavin utilization

The fecal riboflavin excretions on the 5-, 10-, 20-, 50-, 100-, and 1,000-μg. daily doses, were 24.9, 27.8, 33.3, 22.6, 12.0, and 16.4% of the total intake, respectively. The urinary excretions of riboflavin on the same increasing daily doses were 16.6, 7.2, 4.7, 5.5, 11.0, and 9.8%, respectively. The above figures, and table 2 indicate that the low figures for absorbed riboflavin on the 5-, 10-, and 20-μg. daily doses are due to high fecal excretions of this vitamin, most probably due to bacterial synthesis. In this connection, it should be mentioned that no technique has as yet been perfected and, therefore, no precautions were taken to prevent bacterial synthesis while the feces were collecting in the metabolism cages between 5 P.M. and 8 A.M. the next day. That some bacterial synthesis in the feces occurred at night would appear from the fact that for the 5-, 10-, and 20-μg. daily doses of riboflavin, 19.3, 19.7, and 10.3% of the total intake of this vitamin were found in the fecal excretions on a riboflavin-deficient diet; while on the same daily doses of thiamine, 10.0, 9.1, and 3.0% of the total intake of this vitamin were excreted in the feces on a thiamine-deficient ration. With the increase of the daily dose of riboflavin, the amount excreted in the feces on a ration deficient in this vitamin became negligible. On 50-, 100-, and 1,000-μg. daily doses only 3.6, 0.6, and 0.05% of the total riboflavin intake, respectively, were excreted in the feces. The value of the balance studies on these higher doses then becomes relatively much more accurate and it is, therefore, interesting to note that, unlike the metabolism of thiamine, the efficiency of utilization on the large doses remains relatively constant and high. This is particularly true for the 1,000-μg. dose, the efficiency index being 88.3% for riboflavin and only 51.9% for thiamine.

TABLE 2

Influence of increasing doses of riboflavin on efficiency of their utilization.

ANIMAL NUMBER ♂	METABOLISM PERIOD	DAILY RIBOFLAVIN INTAKE	TOTAL RIBOFLAVIN INTAKE	RIBOFLAVIN EXCRETED IN FECES [1]	RIBOFLAVIN ABSORBED		RIBOFLAVIN EXCRETED IN URINE [1]	RIBOFLAVIN RETAINED	RIBOFLAVIN UTILIZED	TOTAL EXCRETIONS OF RIBOFLAVIN IN FECES AND URINE	
	days	*γ*	*γ*	*γ*	*γ*	%	*γ*	*γ*	%	*γ*	%
1	14	5	70	14.9	55.1	78.7	11.5	43.6	79.1	26.4	37.7
2	14	5	70	20.0	50.0	71.4	11.7	38.3	76.6	31.7	45.3
3	13	10	130	31.5	98.5	75.7	9.7	88.8	90.2	41.2	31.2
4	13	10	130	41.2	88.8	68.3	8.9	79.9	90.0	50.1	38.5
5	13	20	260	83.6	176.4	67.8	9.3	167.1	91.2	92.9	35.7
6	13	20	260	89.7	170.3	65.5	14.9	155.4	91.2	104.6	40.2
7	13	50	650	143.5	506.5	77.9	31.7	474.8	93.7	175.2	26.9
8	13	50	650	150.8	499.2	76.7	39.1	460.1	92.2	189.9	29.2
9	21	100	2,100	231.4	1,869.0	89.0	221.9	1,637.0	87.6	453.3	21.6
10	21	100	2,100	270.6	1,829.0	87.1	237.4	1,592.0	87.0	508.0	24.2
11	21	1,000	21,000	3,548.0	17,452.0	83.1	1,942.0	15,510.0	88.8	5,490.0	26.1
12	21	1,000	21,000	3,356.0	17,644.0	84.8	2,148.0	15,496.0	87.8	5,504.0	26.2

[1] Corrected for the amounts excreted on a riboflavin-deficient ration.

Since the excretions of thiamine and riboflavin in the feces and urine do not account for their total intake, the suggestions, of course, present themselves that excess of these vitamins are either stored in the tissues or destroyed during metabolism. To test the first hypothesis, two groups of male rats, six in each group, each about 35 days old and weighing approximately 100 gm., were given two different doses of thiamine and riboflavin for 30 days, supplementing the synthetic diet,

TABLE 3

Food consumption and changes in body weights on increasing doses of thiamine and riboflavin.

th.n.c. = thiamine negative control.
rf.n.c. = riboflavin negative control.

ANIMAL NUMBER (MALES)	DAILY DOSE OF THIAMINE	DAILY DOSE OF RIBOFLAVIN	METABOLISM PERIOD	TOTAL FOOD CONSUMPTION	CHANGE IN BODY WEIGHT
	μg.	*μg.*	*days*	*gm.*	*gm.*
1	5	5	26	178.9	+ 14
1 — th.n.c.	0	5	26	125.3	− 1
2	5	5	26	193.9	+ 31
2 — rf.n.c.	5	0	26	156.8	− 12
3	10	10	13	147.1	+ 35
3 — th.n.c.	0	10	13	134.9	+ 39
4	10	10	13	148.3	+ 44
4 — rf.n.c.	10	0	13	113.9	+ 21
5	20	20	13	139.3	+ 43
5 — th.n.c.	0	20	13	104.2	+ 29
6	20	20	13	156.0	+ 39
6 — rf.n.c.	20	0	13	122.1	+ 27
7	50	50	13	132.7	+ 31
7 — th.n.c.	0	50	13	122.8	+ 20
8	50	50	13	128.4	+ 25
8 — rf.n.c.	50	0	13	94.6	+ 9
9	100	100	26	296.9	+ 97
9 — th.n.c.	0	100	26	130.9	− 18
10	100	100	26	305.7	+ 88
10 — rf.n.c.	100	0	26	182.1	+ 31
11	1,000	1,000	26	345.5	+ 115
11 — th.n.c.	0	1,000	26	111.6	− 13
12	1,000	1,000	26	312.8	+ 97
12 — rf.n.c.	1,000	0	26	173.0	+ 11

composition of which is given earlier in the paper; in addition, each animal received daily 20 μg. pyridoxine, 6 mg. choline chloride, and 200 μg. calcium pantothenate, One group received 100 μg. thiamine and 100 μg. riboflavin per animal per day and in the other group 1,000 μg. thiamine and 1,000 μg. riboflavin were given daily to each animal. At the end of the experiment the various tissues were dissected out, dried at 55° C. and analyzed for thiamine and riboflavin, according to techniques recently described (Sure and Ford, '41). It will be noted from table 4 that the concentration of these vitamins,

TABLE 4

Influence of 100 μg. and 1,000 μg. daily administration of thiamine and riboflavin for 30 days on distribution of these vitamins in various tissues.

TISSUE	THIAMINE		RIBOFLAVIN	
	100 μg. thiamine and 100 μg. riboflavin daily	1,000 μg. thiamine and 1,000 μg. riboflavin daily	100 μg. thiamine and 100 μg. riboflavin daily	1,000 μg. thiamine and 1,000 μg. riboflavin daily
	μg./gm.	*μg./gm.*	*μg./gm.*	*μg./gm.*
Liver	8.60	8.10	47.50	36.25
Kidney	9.70	8.40	62.50	66.25
Spleen	3.73	3.40	10.63	16.88
Heart	2.50	3.29	20.83	20.84
Lung	2.00	1.80	10.63	8.75
Brain	2.55	4.60	8.54	12.08
Testes	4.60	5.40	8.75	15.00
Stomach	2.75	1.35	6.25	7.92
Small intestines	1.80	1.80	12.50	6.57
Large intestines	2.87	1.37	7.50	7.50
Pancreas	1.76	1.20	8.84	10.00
Muscle	0.63	1.80	1.67	2.08

expressed as micrograms per gram on the dry basis, is not appreciably greater on the 1,000-μg. daily dose contrasted with the 1000-μg. daily allowance. In some tissues higher results are even indicated on the lower dose. In this respect, our findings are in accord with those of Leong ('37) who reported that maximum storage takes place on 90 μg. per day of vitamin B_1 intake. We found, however, considerably smaller excretions of thiamine in urine and much greater excretions of this vitamin in the feces on as high doses as 1,000 μg. daily;

also, we found that the fecal thiamine begins to rise on the 20-μg. rather than on the 150-μg. daily dose. In order to obtain information on the total thiamine and riboflavin stored in the body tissues, six additional male rats of similar age and weights as used in the other experiments outlined above were given 1,000 μg. thiamine and 1,000 μg. riboflavin daily for 30 days. They were then sacrificed and these vitamins were determined in their entire bodies. The results are given in table 5. It will be noted that after the intake of 30,000 μg. and an

TABLE 5

Thiamine and riboflavin content of animals which received 1,000 μg. of these vitamins daily for 30 days.

		THIAMINE		RIBOFLAVIN	
Animal number and sex	Dry weight of animal	μg./gm.	Thiamine content of animal	μg./gm.	Riboflavin content of animal
5290 — ♂	39.00	2.16	84.24	18.00	702.00
5290 — L♂	46.28	2.82	130.51	21.00	971.88
5291 — ♂	42.32	3.33	140.92	17.50	740.60
5292 — ♂	40.12	2.60	104.31	17.00	682.04
5292 — L♂	44.99	2.71	121.92	17.00	764.53
5302 — ♂	48.03	3.47	166.66	17.50	840.53
Average			124.76		782.64

equal amount of riboflavin, the average content per animal in its entire body was only 125 μg. thiamine and 783 μg. riboflavin. From such results it is quite apparent that the animal organism has only a very limited capacity for storing these two components of the vitamin B complex. How then can we account for the tremendous wastes in the metabolism of thiamine and riboflavin, particularly on the higher doses of intake? If oxidative or other destructive processes occur in the tissues, how could that be demonstrated experimentally? Although, in vitro we can never imitate vital phenomena occurring in vivo, attempts were made to determine if incubation at 37°C., or body temperature, of these vitamins with various body tissues for 24 to 48 hours, would show any destruction. Our results, presented in table 6, show appreciable destruction of

both thiamine and riboflavin by liver, lung, heart, stomach, small and large intestines. From the above results, it would appear then that at least an appreciable proportion of the losses of thiamine and riboflavin in metabolism is caused by their destruction in the body tissues after they have been utilized by such tissues for various functions.

TABLE 6

Influence of incubation at 37° C. on destruction of thiamine and riboflavin by body tissues.

Amount of thiamine incubated — 1000 μg.
Amount of riboflavin incubated — 1000 μg.

TISSUES	THIAMINE		RIBOFLAVIN	
	Incubation period		Incubation period	
	24 hours	48 hours	24 hours	48 hours
	Per cent destruction	*Per cent destruction*	*Per cent destruction*	*Per cent destruction*
Liver	0.0	14.0	5.0	20.0
Kidney	1.0	0.0	0.0	3.0
Spleen	7.0	...	7.5	25.0
Lung	8.5	28.0	25.0	25.0
Heart	10.5	17.0	28.0	25.0
Brain	1.0	...	0.0	10.0
Stomach	5.0	12.0	0.0	20.0
Small intestines	20.0	26.0	12.0	20.0
Pancreas	4.0	20.0	8.0	25.0
Thymus	0.0	...	0.0	...
Adrenals	17.0	...	0.0	...
Thyroids	0.0	...	12.5	...
Pituitary	6.0	...	2.5	...

SUMMARY

A quantitative metabolism study was made of the influence of increasing doses of thiamine and riboflavin on their efficiency of utilization. The daily doses used were: 5, 10, 20, 50, 100, and 1,000 μg. For thiamine, the 5-, 10-, 20-, and 50-μg. doses were most efficiently utilized. On higher levels of intake the efficiency of utilization decreases, there being a marked drop on the 1,000-μg. daily dose. The efficiency of utilization on the 50-, 100-, and 1,000-μg. daily doses were 92.1, 82.9, and 51.9%, respectively.

It is impossible at present to evaluate the efficiency of utilization of the 5-, 10-, and 20-μg. doses of riboflavin, because the fecal excretions indicate some bacterial synthesis. However, on the higher intakes of 50-, 100-, and 1,000-μg. doses, the total fecal riboflavin excretions are insignificant in terms of the total intake of this vitamin, hence the figures of 76 to 88% utilization on these higher doses are significant, and show that massive doses of riboflavin are much more economically utilized than equal intakes of thiamine. The total thiamine and riboflavin excretions do not account for the total intake of these vitamins. Neither could they be accounted for by storage in the animal tissues. However, incubation at 37° C. for 22 to 48 hours showed appreciable destruction of thiamine and riboflavin by liver, lung, heart, stomach, small and large intestines. It would appear then that part of the losses of thiamine and riboflavin in metabolism are caused by their destruction in the tissues.

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STUDIES ON CAROTENOID METABOLISM

IV. THE EFFECT OF VITAMIN A INTAKE ON THE CAROTENOID AND VITAMIN A CONTENT OF THE EGGS, LIVER, BLOOD AND BODY FAT OF HENS

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TWO FIGURES

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Not only has it been shown that the transfer of vitamin A to the milk is greatly augmented when massive doses of this vitamin are administered to cows, but also it has been demonstrated that this is accompanied by a concomitant decrease in the butter carotene in spite of the fact that the intake of the carotenoids remains essentially constant (Deuel, Halliday, Hallman, Johnston and Miller, '41; Deuel, Hallman, Johnston and Mattson, '42). In a later study, Mattson and Deuel ('43, a) found that a similar depression in blood and liver carotenoids accompanied the administration of large doses of vitamin A in baby chicks although the pigments here are chiefly the carotenols such as lutein (xanthopyll). In the present study, the investigations have been extended to adult chickens to determine whether an increased vitamin A intake would result in an augmented excretion of vitamin A in the eggs along with a decreased pigment content. Studies have also been made of the egg production and hatchability of the eggs produced on the several diets which will be reported elsewhere.

In eggs vitamin A is present almost entirely in the yolk. The quantity has been shown to vary with diet but the average level is given by Sherman ('41) as 2,500 to 5,000 I. U. per 100 gm. of yolk which would give an average value of 600 I. U. (400 — 800 I. U.) per egg where the yolk weighs approximately 16 gm. Russell and Taylor ('35) report values of 500 to 800 I. U. per 16 gm. yolk in eggs obtained from chickens on several diets. Other results based on bioassays are somewhat lower. Ellis et al. ('32) report values of about 500 Sherman-Munsell units per egg (31 S. M. units per gram yolk) in a diet fortified with cod liver oil while Koenig et al. ('35) give values averaging 33 and 20 S. M. units per gram of yolk in low and high egg producers respectively. Pale yolked eggs on carotene-free diets containing cod liver oil contained 25 S. M. units per gram of yolk. Bearse and Miller ('37) obtained values of 10 to 28 I. U. per gram of yolk, the higher levels being found with an increased intake of vitamin A. Sjollema and Donath ('40) noted a maximum of 321 I. U. per egg in chickens fed on a yellow corn-alfalfa diet although this was lowered approximately 50% when the alfalfa was omitted. Cruickshank and Moore ('37) have found that the A content of eggs was doubled by the incorporation of 10% of cod liver oil while it was increased five times by the administration of an A concentrate. The augmentatory effect of diet is also reported by Sherwood and Fraps ('34) and Bethke, Kennard and Sassaman ('27). Hauge and Zscheile ('42) have shown that the vitamin A content of eggs dried by the Mojonnier spray drier is destroyed less than 5%; they report a value of 44 I. U. per gram of dried whole egg which would correspond with approximately 33 I. U. per gram of yolk.

The effect of the carotenoid intake on the color of egg yolk has been recognized since the classical work of Palmer and Kempster ('19). More recently it has been shown that the amount of carotenols deposited varies with the source of the xanthopylls as well as with the amount ingested. Also the content of carotene and cryptoxanthine was found to vary with different hens and it could not be increased with in-

creased intake (Hughes and Payne, '37). In fact only 2.5% of the carotene in alfalfa was shown to be deposited in the yolk. Sjollema and Donath ('40) found that the carotene made up only approximately 7% of the total carotenoids in the yolks of an alfalfa or yellow corn-alfalfa diet while it comprised an average of 11% on a diet in which the pigments were derived only from yellow corn.

On the other hand the depressing effect of a very high intake of vitamin A on the pigmentation of egg yolks has not been recognized. It is demonstrated here that this factor may have as profound an effect as that of the exogenous source of these pigments.

EXPERIMENTAL

The experiments were carried out on six groups of white Leghorn chickens each of which was made up of twenty-seven hens and three roosters. With two groups of chickens, the vitamin A supplements were increased after 3 months so that a control group and seven levels of vitamin A feeding are reported here. They were maintained throughout on a basal diet high in carotenoids which had the following composition per 100 lbs:

Ground yellow corn	34.90 lbs.
Recleaned ground oats	5.00 lbs.
Ground wheat	24.90 lbs.
Flaky white bran	17.95 lbs.
Fish meal	5.48 lbs.
Dehydrated alfalfa	5.00 lbs.
Soybean meal	2.50 lbs.
Dried whey	2.00 lbs.
Oyster shell flour	1.00 lbs.
Fine oyster shell grit	0.745 lbs.
Sodium chloride	0.50 lbs.
Manganese sulfate	0.025 lbs.

To this was added 22,500 units of vitamin D, (50 units per 100 gm. of feed).

The basal diet was fed to all the groups during the basal period and to the control group throughout the experiment. It contained approximately 10 mg. of carotenoids per pound

of food. Sufficient high potency shark liver oil (approximately 20,000 I. U. per gram) was mixed with the basal diet to give the food an additional potency of 1,000, 2,000, 15,000, 30,000, 60,000, 100,000, and 200,000 I. U. per pound. To avoid loss of vitamin A by prolonged storage, the diets were made up at frequent intervals by mixing the basal diet and the required amount of vitamin A oil. The food consumption of the chickens approximated ¼ lb. per day; therefore the average daily intake of vitamin A per chicken was approximately one-fourth of the above values. The basal period began on August 21, 1942 and continued until November 2nd except with group 7 where it was terminated on October 15th. At the termination of the basal period, the supplements were mixed with the diets and fed until the termination of the experiment on April 24, 1943. After 75 days on the supplement, groups 3 and 4 were discontinued, and two new groups were formed (6 and 8) by placing approximately 50% of the chickens in each of the original groups in each of the new groups. These were continued on the new levels of vitamin A (60,000 and 200,000 I. U.) until the termination of the test.

The tests were carried out at the poultry ranch associated with the Emeryville Plant of the California Packing Corporation and were supervised by one of us (A. R.). Twelve eggs from each group from the previous days' production were forwarded to Los Angeles by express each Saturday and analyses were usually started on the following Tuesday.

The analyses for vitamin A and carotenoid pigments were made on aliquots from the pooled samples of the yolks from each group. After determining the total weights of the whole egg, the yolks were separated mechanically, weighed, and homogenized in a Waring blendor. Weighed samples were saponified in 50% alcohol with 5 ml. of 40% KOH for 30 minutes and then transferred to an all glass extraction apparatus where they were extracted for 3 hours with low boiling Skelly Solve. Saponification and extraction were carried out in a dark room where the light globes were covered with a special lacquer [1]

[1] This lacquer was kindly furnished us by Mr. A. Cherkin of the laboratories of Don Baxter, Inc., Glendale, California.

inasmuch as it has been shown that marked destruction of vitamin A takes place not only in direct sunlight but also in diffuse daylight or even in light from mazda lamps (Mattson and Deuel, '43, b). The petroleum ether extract was evaporated to small volume, filtered into 50 ml. volumetric flasks, absolute isopropanol was added and the final volume of solvents adjusted so that the concentration was 75% isopropanol and 25% petroleum ether. The absorption was determined on the Beckman spectrophotometer at 326 mμ (for vitamin A) and 446 mμ (for carotenoids). The value for absorption of vitamin A was corrected for that of the carotenoids by multiplying $\log. \frac{I_0}{I_{446}}$ by the factor 0.06. A conversion factor of 2060[2] was used to determine I. U. of vitamin A per gram of yolk from the extinction coefficients.[3]

Since xanthopyll makes up the largest portion of the carotenoids, the concentration of total carotenoids was calculated from the absorption coefficient at 446 mμ divided by 0.252[4] which was determined on a pure lutein sample[5]. In cases where vitamin A was determined on single eggs, a homogenous suspension was made with 95% alcohol in the Waring blendor and appropriate aliquot samples were used for saponification.

At the conclusion of the experiments, six hens of each group were killed by bleeding following a 72-hour fast. Determinations of carotenoids and vitamin A were made on the blood plasma, liver and body fat. The liver was homogenized with 95% ethyl alcohol in the Waring blendor and stored several days in the refrigerator until determinations could be made. No decrease was noted in samples kept for a week under such conditions.

[2] The spectrophotometer was checked at frequent intervals with potassium chromate (Morton, '42). The factor, 2060, was obtained by calibration with reference cod liver oil.

[3] $\text{Vitamin A}_{\text{(gm. yolk)}} = \dfrac{(\log \frac{I_0}{I_{326}} - 0.06 \log \frac{I_0}{I_{446}}) \times \text{dilution}}{\text{Wt. of aliquot}} \times 2060$

[4] $\text{Carotenols per gram yolk} = \dfrac{\log \frac{I_0}{I_{446}} \times \text{dilution}}{\text{Wt. of aliquot} \times 0.252}$

[5] This sample of pure lutein was kindly furnished us by Dr. L. Zechmeister of the California Institute of Technology.

RESULTS

The average values for vitamin A and carotenoids in the yolks obtained over the 24-week period are given in table 1 while the fluctuations are evident from figures 1 and 2.

There was no alteration in pigment content of the yolks where 1,000 or 2,000 I. U. of vitamin A were incorporated per pound of food. A definite decrease was found in group 4 (15,000 I. U. per pound) and a progressively greater decrease with succeeding groups to 25% of the original level with the highest intake of vitamin A (group 8).

On the other hand, no increase in the vitamin A content of eggs obtained in group 4 where a suppressing effect on the carotenoid pigments had been noted. A small increase was found in group 5 and a gradual rise in the grand average to 121 I. U. per gram of yolk for group 8. During the course of the experiments, averages during individual weeks as high as 158 I. U. were found; in the last week of the test, where determinations were made separately on eggs from ten different chickens in group 8, an average of 141.7 I. U. was noted, the maximum being 226.3 and the minimum 91.8 I. U. per gram of yolk. The total carotenoids in separate eggs averaged 8.17 micrograms per gram of yolk, the variations being from 14.1 to 3.55 micrograms.

The results of the analyses of the liver, plasma, and body fat of six chickens from each group (except groups 3 and 4) are recorded in table 2.

The carotenoids in the serum and liver are apparently progressively lowered in groups 5 to 8 over the control group and the group receiving 1,000 I. U. per gram. Because of the small number of observations, these results are statistically significant only with the two groups receiving the highest level of supplement. On the other hand, the carotenoids show a marked lowering in the body fat only in group 7; in group 8, where the suppression of carotenoids in the liver and serum had been the greatest, no decrease was noted.

The vitamin A content of the serum is constant although there is some indication that it may be somewhat elevated

TABLE 1

The carotenoid and vitamin A contents of eggs during a basal period (Period I) when only the basal diet was fed and during an experimental period (Period II) when different amounts of vitamin A were incorporated in the diet.

GROUP NO.	VITAMIN SUPPLEMENT PER POUND FOOD	NO. OF SAMPLES		CAROTENOIDS IN YOLK			VITAMIN A IN YOLK			VITAMIN A PER EGG (18 GM. YOLK)	
		Period I	Period II	Period I	Period II		Period I	Period II		Period I	Period II
					Amount [1]	M.D.: S.E.M.D.[2]		Amount [1]	M.D.: S.E.M.D.[2]		
	I. U.			γ/gm.	γ/gm.		*I. U./gm.*	*I. U./g.m*		*I. U.*	*I. U.*
1	0	3	23	38.3	32.3 ± 2.0		46.6	6.8 ± 0.7		840	844
2	1,000	3	23	36.1	29.4 ± 1.5	1.16	44.6	46.7 ± 0.8		803	842
3	2,000	3	9	40.2	33.0 ± 1.7	0.27	46.8	46.3 ± 1.3		844	835
4	15,000	3	11	36.8	25.3 ± 0.7	3.30	47.9	48.4 ± 0.7	1.62	862	872
5	30,000	2	23	39.0	21.7 ± 1.1	4.65	48.6	54.6 ± 0.8	7.35	875	982
6	60,000[3]	..	12	(29.2)	12.9 ± 0.8	9.12	(47.4)	62.3 ± 3.3	5.56	(854)	1122
7	100,000	3	24	38.0	9.7 ± 0.6	10.82	44.3	99.3 ± 3.3	15.53	798	1785
8	200,000[3]	..	13	(29.2)	8.4 ± 0.5	11.62	(47.4)	120.7 ± 7.1	10.38	(854)	2170

[1] Including the standard error of the mean calculated as follows: $\sqrt{\Sigma d^2/n}/\sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations. These averages for carotene in all groups on Period II and for vitamin A in groups 6, 7 and 8 exclude the values for the first 3 weeks of supplement.

[2] Mean Difference: Standard Error of Mean Difference calculated as follows: $M.D./\sqrt{(S.E.M._1)^2 + (S.E.M._2)^2}$. When this exceeds 3.00 the results are considered significant. The comparisons are made in all cases with the control group.

[3] The values in parentheses are the average values of groups 3 and 4 for the 8-week period immediately preceding. After that groups 6 and 8 were started with the hens comprising those of groups 3 and 4.

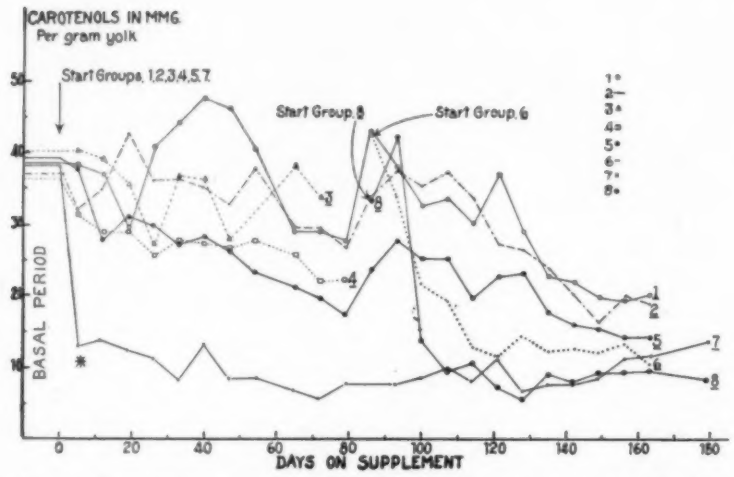

Fig. 1 The carotenol content in micrograms per gram of yolk. The first measurement (starred) in the curve for group 7 only was made at an interval of 23 days after the start of the supplement.

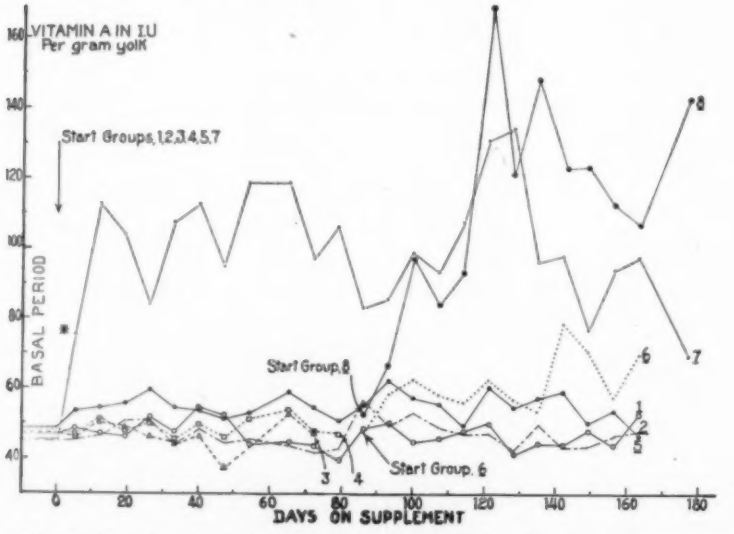

Fig. 2 The vitamin A in I.U. per gram of yolk. The first measurement (starred) in the curve for group 7 only was made at an interval of 23 days after the start of the supplement.

in group 8. On the other hand, there seems to be no further increase in storage of vitamin A in the liver when the supplement is greater than 30,000 I. U. of vitamin A although the maximum values become progressively higher. The maximum storage was noted in one of the hens of group 7 where 411,000 I. U. were stored in the whole liver.

The body fat shows a marked rise in vitamin A storage which, however, is only slight in the 30,000 I. U. group but reaches an average about eight times the basal level in the 200,000 I. U. group. In one chicken here a maximum vitamin A content of 340 I. U. was found per gram of fat compared with an average value for the control group of 32.3 I. U. per gram.

The hens were in good condition throughout the test although those receiving the high levels of vitamin A had a different appearance because of the loss of carotenols from the

TABLE 2

The carotenoid and vitamin A content of the serum, liver and body fat of hens which had received the basal diet only for 8 months (group 1) or with added vitamin A supplements for approximately 3 months (groups 6 and 8) or for approximately 6 months (groups 2, 5 and 7).

GROUP NO.[1]	BODY WEIGHT	CAROTENOIDS			VITAMIN A		
		Serum	Liver	Fat	Serum	Liver	Fat
	gms.	*γ/100cc.*	*γ/gm.*	*γ/gm.*	*I. U./gm.*	*I. U./gm.*	*I. U./gm.*
1	1551	143.2[2]	7.8	9.5	463	370	32.3
		(212.8–60.3)	(14.3–6.0)	(15.8–4.5)	(550–372)	(943–78)	(52.4– 19.4)
2	1603	218.0	11.7	4.5	554[2]	665	23.4
		(501.5–31.8)	(21.3–5.4)	(6.7–0.0)	(804–346)	(1013–70)	(37.7– 15.5)
5	1635	111.4	6.7	5.9	445	4592	42.2
		(183.1–51.8)	(11.7–4.4)	(7.8–3.0)	(517–388)	(9043–406)	(58.3– 32.6)
6	1656	105.8	6.3	6.1	580	6940	59.3
		(163.5–29.6)	(10.6–2.2)	(8.1–4.5)	(1320–350)	(10070–2780)	(77.0– 41.6)
7	1585	62.6	4.0	2.3	443	4654	92.7
		(205.2–11.8)	(8.2–1.5)	(5.1–0.3)	(650–262)	(12825–1100)	(166.0– 25.0)
8	1602	37.0	2.7	8.0	689	4626	222.6
		(68.6–11.6)	(4.5–0.8)	(16.1–2.8)	(1076–464)	(13888–1220)	(340.0–167.6)

[1] Six chickens in all groups except group 5 where there were five. The figures in parentheses represent the maximum and minimum values in each group.

[2] Five determinations only.

combs and wattles. There was no evidence of an adverse effect on egg production; in fact, there is some indication of an increased production although the experiments are not sufficiently extensive to make this certain. No gross differences in food consumption were noted.

DISCUSSION

Large doses of vitamin A depress the carotenol content of hen's egg in the same way that the carotene content of cow's milk is lowered (Deuel et al. '42). In the experiments on cows, a daily intake of 230,000 or 470,000 I. U. of vitamin A brought a lowering in carotene although no rise in vitamin A content of the milk obtained. In the case of hens a similar phenomenon was noted; the carotenoid content of the eggs of group 4 which received 15,000 I. U. of vitamin A per pound of food was significantly lowered although the vitamin A in the eggs was not increased.

The carotenol content of the serum is also progressively lower with increasing doses of vitamin A in the diet. No marked effect was noted in the liver, however, except with groups 7 and 8 although the results on groups 5 and 6 might be significantly lower if more experiments were available. Also, the most marked lowering in carotenoid content of body fat obtained in group 7 where the values are about 25% of the levels found in the control group. The mean figure for group 8, however, shows no lowering from the control level although this group had exhibited the greatest lowering in the pigment content of the serum and liver. The discrepancy between groups 7 and 8 may be explained because of the difference in the length of time during which the supplement was administered. The analyses on group 7 were obtained from tissues of chickens which had received the vitamin A supplement for 6½ months while the results on group 8 were obtained after the birds had received the food containing 200,000 I. U. of vitamin A per pound for only 3 months. Prior to that they had been receiving supplements of 2,000 or 15,000 I. U. per pound. These data would seem to illustrate the slow

turnover of the carotenoids in body fat as contrasted with the much more rapid response of the liver, serum and eggs.

The vitamin A content of the eggs was slightly increased in the 30,000 I. U. group and progressively higher as the supplements were increased. The maximum average value of 158 I. U. per gram of yolk found in group 8, corresponds with 2,840 I. U. of vitamin A per egg. The highest value of 226 I. U. found in a single egg in group 8 accounts for 3,850 I. U. of vitamin A. Still a higher concentration was obtained in an ovum from one of the hens which was sacrificed where a value of 250 I. U. of vitamin A per gram was noted.

The maximum lowering in carotenols requires 20 to 30 days and continues to be maintained for at least 6 months. About the same interval is required for the vitamin A to reach the higher level in the yolk. There is a marked fluctuation in the mean value obtained on different weeks after the higher level has been reached. This may be due to an alteration in the vitamin A deposited or to the variation in the sources of the eggs at different times. Since it is evident from our examination of the eggs of different hens that a marked variation may be obtained, a difference in the mean value may very well be caused by the inclusion or exclusion of some of the eggs containing the maximum levels of vitamin A. There is no evidence that a tolerance for ingested vitamin A may develop in 6 months with a resultant drop in the vitamin A excreted in the egg.

SUMMARY

The administration of large amounts of vitamin A to hens receiving a basal diet high in carotenoids resulted in a marked and progressively greater suppression in the pigment content of the egg yolk as the vitamin A intake was increased. On the diet in which 15,000 I. U. of vitamin A was incorporated per pound, the lowering was significant, while it was decreased to only 25% of the basal level in chickens receiving the basal diet containing 200,000 I. U. of vitamin A per pound of food. A similar effect was noted in the blood and liver; it was also strikingly evident in the body fat of group 7 which

had received a supplement of 100,000 I. U. of vitamin A for over 6 months but not in the case of group 8 which received twice the level of vitamin A for 3 months. This is considered to be the resultant of the sluggish turnover of the carotenoids in body fat.

The vitamin A in the yolk was unchanged from the average control level of 46.8 I. U. per gram when 1,000, 2,000 or 15,000 I. U. of this vitamin were added per pound of food. With higher doses of vitamin A in the diet, significantly increased levels were found in the yolks, the values respectively being the following: 30,000 I. U. group, 54.6; 60,000 I. U. group, 62.3; 100,000 I. U. group, 99.3 I. U.; and with the 200,000 I. U. group, 120.7 I U. The maximum level found was 226 I. U. There was no evidence of the development of a tolerance for vitamin A with a resultant decrease in the vitamin A excreted during a 6-month period.

The vitamin A is progressively increased in the body fat with the higher intake in the diet. This was augmented from a value of 32.3 I. U. per gram in the control group to a mean value of 222.6 I. U. per gram in the group receiving 200,000 I. U. per pound of food. Maximum levels of vitamin A in the liver were found in the 60,000 I. U. group and no greater increases were noted when the intake was further augmented. The vitamin A in the serum was unaltered except that a slight increase probably occurred when the supplement was fed at the highest level.

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BORDEN AWARD IN NUTRITION

The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time. The award will be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by February 1, 1944. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

HENRY A. MATTILL,
State University of Iowa,
Iowa City, Iowa

Chairman, Nominating Committee

MEAD JOHNSON AND COMPANY
'B-COMPLEX' AWARD

Nominations are solicited for the 1944 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B Complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1943 must be in the hands of the Secretary by January 10, 1944. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

ARTHUR H. SMITH
Wayne University College of Medicine
Detroit, Michigan

SECRETARY, AMERICAN INSTITUTE OF NUTRITION

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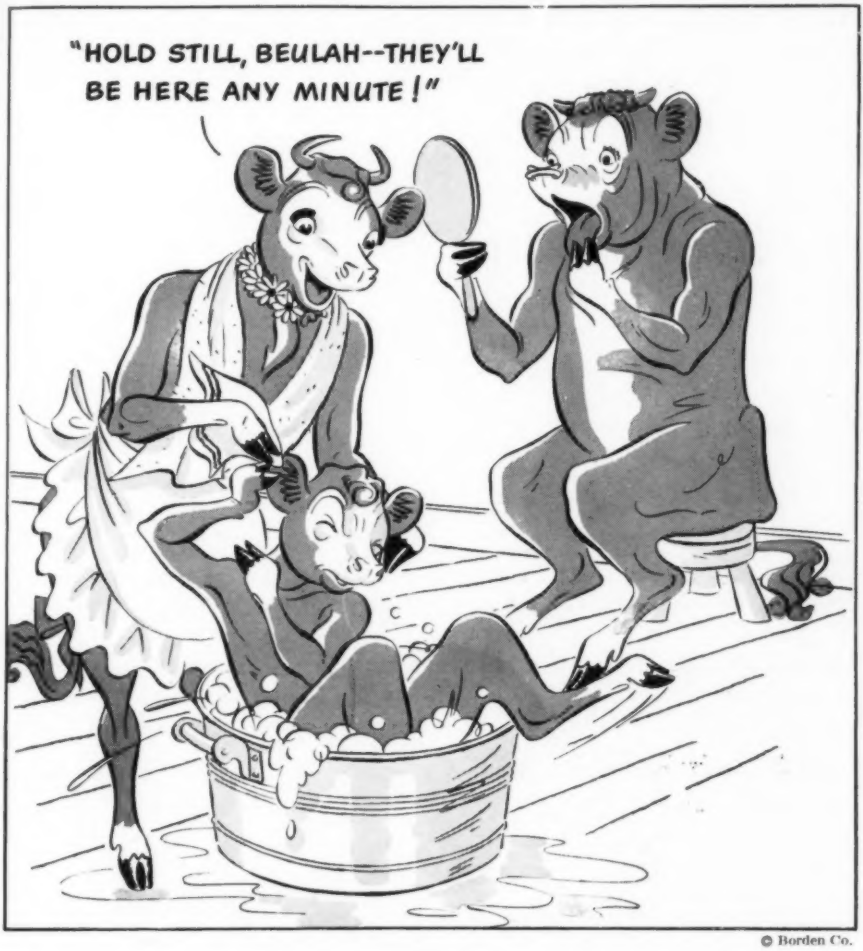

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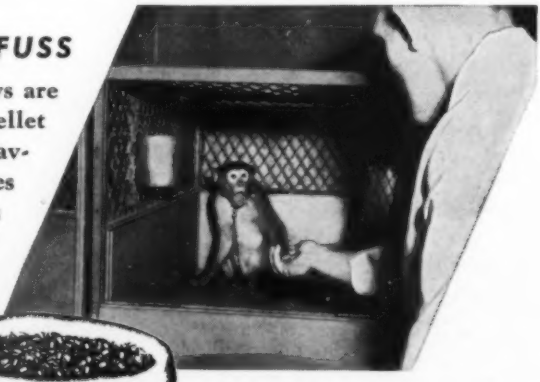

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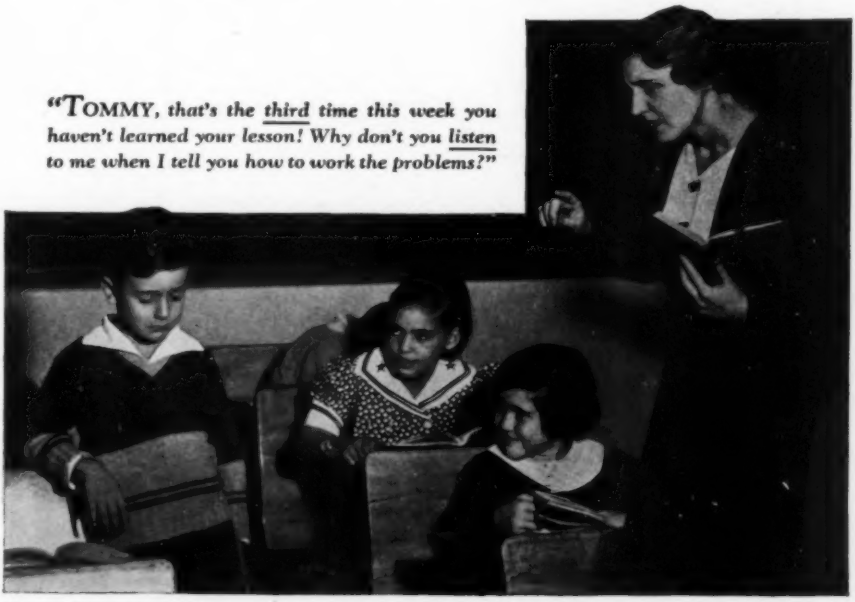

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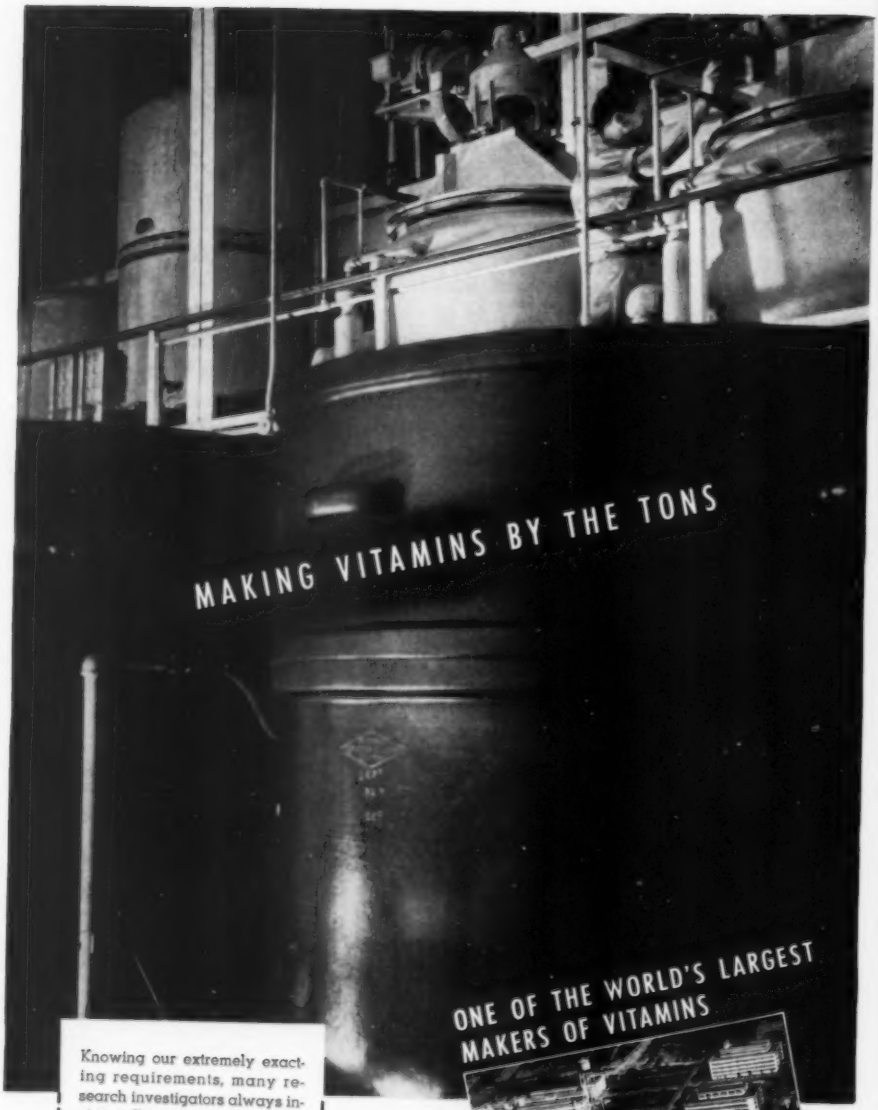

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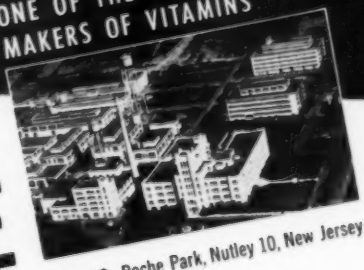

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